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# TECHNICAL EVALUATION OF SAMPLE-PROCESSING, COLLECTION, AND PRESERVATION METHODS

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To determine the identity of a potential threat, an essential step includes the reliable isolation and purification of unique nucleic acids from unknown agents. The ability to provide polymerase chain reaction-ready DNA is necessary for the positive identification of unknown biological threats. At the time of this study, there were several commercially available assays for isolating and purifying nucleic acids from potential threat agents, but these assays were designed to isolate nucleic acids either for specific types of pathogens or from predetermined matrices. There were no commercially available nucleic acid preparation assays that had the flexibility and broad scope necessary for the wide variety of samples and sample matrices that can be encountered in a biodefense or first-responder setting. It was important to evaluate the available technologies and determine which preparation, collection, and preservation method(s) would be most useful in an emergency setting. This study provided an unbiased technical evaluation of commercially available nucleic acid preparation assays, collection methods and devices, and preservation methods and devices that could be used to support Department of Defense and other interagency acquisition decisions. This study was divided into three specific subsections: (1) sample preparation, (2) sample collection, and (3) sample preservation.

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# TECHNICAL EVALUATION OF SAMPLE-PROCESSING, COLLECTION, AND PRESERVATION METHODS

## 1. INTRODUCTION

### 1.1 Policy

*The National Strategy for Combating Biological Threats* (National Security Council, 2009) outlined a broad framework defining the needs and critical parameters involved in the constant struggle against naturally occurring infectious diseases, food and environmental safety, and potential intentional harmful use of microorganisms. This strategy document called for increased international cooperation, focus on laboratory safety and security, better detection and countermeasure capabilities, and transparent communications. More recently, an adjunct policy document entitled *The National Strategy for Biosurveillance* was released (White House, July 2012) as part of the National Security Strategy. This document provides additional guidance for the specific aims, goals, and methods by which the U.S. Government will implement systems to provide enhanced situational awareness of biological threats to the environment, human health, agriculture, and food supplies. Specifically mentioned is the concept of leveraging existing capabilities to "scan and discern the environment," which implies the use of current technical biosurveillance capabilities that span the spectrum from sample processing to handheld point-of-use detectors to more sophisticated, laboratory-based instrumentation with high sample throughput and data resolution.

### 1.2 Support for Joint Program Executive Office for Chemical Biological Defense/U.S. Government (JPEO-CBD/USG) Acquisition Programs

The continuing mission of the Joint Project Manager Medical Countermeasure Systems (JPM-MCS; formerly Joint Project Manager Transformational Medical Technologies [JPM-TMT]) includes the ability to counter new, emerging, and engineered infectious agents. The goal of the Response Systems program within JPM-MCS is to provide a rapid and robust detection capability, starting with sample processing, that will provide the warfighter and interagency partners with the ability to detect and identify infectious diseases and engineered threats at the point of contact. To facilitate acquisition programs in support of the Department of Defense and interagency missions, technical paper studies and laboratory testing of existing sample-processing technologies are expected to enable in silico evaluations of biosurveillance methodologies, equipment, and technologies. This data will allow procurement managers to assign variable weights to multiple technical and performance factors before making their decisions. This will allow for an analysis of the alternatives and rational decision making when managers allocate advanced development and acquisition funding.

### 1.3 Sample-Processing Evaluation Project Overview

Terrorist attacks, such as the anthrax mailings in 2001, have shown the importance of preparing for the possibility of biologically based attacks on military, civilian, or agricultural targets. To be fully prepared for this possibility, it is essential to have the ability to

consistently and rapidly identify potential biological threats. The reliable isolation and purification of unique nucleic acids from unknown agents includes providing polymerase chain reaction (PCR)-ready DNA as a necessary step for the positive identification of unknown biological threats. At the time of this report, there were several commercially available assays for isolating and purifying nucleic acids from potential threat agents. However, these assays were designed to isolate nucleic acids for either specific types of pathogens (e.g., Gram-positive or Gram-negative bacteria) or from predetermined matrices (e.g., blood, soil, etc.). There were no commercially available "gold-standard" nucleic acid preparation assays that had the flexibility and broad scope necessary for the wide variety of samples and sample matrices that are encountered in a biodefense and first-responder setting. It is very important to evaluate the currently available technologies and determine which preparation, collection, and preservation method(s) will be most useful in an emergency setting. This study provided an unbiased technical evaluation of commercially available nucleic acid preparation assays, collection methods and devices, and preservation methods and devices that could be used to support Department of Defense and other interagency acquisition decisions. This study was divided into three specific subsections: (1) sample preparation, (2) sample collection, and (3) sample preservation.

During the sample-preparation study, the staff from the BioSensors Branch of the U.S. Army Edgewood Chemical Biological Center (ECBC) evaluated 20 commercially available nucleic acid isolation assays for their effectiveness when used for DNA isolation for two well-described biodefense-specific agents. These agents included a Gram-positive organism (*Bacillus atrophaeus* var. *globigii*) and a Gram-negative organism (*Yersinia pestis* CO92 pigmentation negative [pgm–] vegetative cells). The nucleic acid preparation assays used in this study included both filter-based and bead-based DNA isolation technologies. In addition to this initial evaluation, soil samples (clay and sand) spiked with *B. atrophaeus* var. *globigii* spores were evaluated with six soil-specific DNA extraction assays. Each method was evaluated on the following seven criteria: (1) preparation time, (2) cost per sample, (3) ease-of-use, (4) resultant DNA quality, (5) percent DNA recovery, (6) effectiveness of PCR, and (7) end-users reviews. Tests were performed in an ISO/IEC 17025-compliant laboratory (International Organization for Standardization [ISO]/International Electrochemical Commission [IEC], 2005). Results for each DNA extraction assay are reported in Section 3.

During the sample-collection study, the BioSensors Branch personnel evaluated six commercially available sample-collection devices for their ability to be used for the recovery of *B. atrophaeus* var. *globigii* spores from several different standardized surfaces (e.g., glass, stainless steel, sealed concrete, treated wood, and carpet). Collection devices were initially evaluated on their ability to be used to recover viable bacteria. In addition, DNA obtained from the various collected samples was extracted using an identical process—the Blood and Tissue Midi Preparation Kit (Qiagen, Inc.; Valencia, CA)—and evaluated for the following criteria: (1) resultant DNA quality; (2) percent DNA recovered; and (3) effectiveness of the recovered DNA in PCR analysis. These tests were also performed in an ISO/IEC 17025-compliant laboratory. The results for each collection device are reported in Section 4.

In the sample-preservation study, the BioSensors Branch personnel evaluated four commercially available sample-preservation methods for their ability to be used to preserve

isolated DNA from *Y. pestis* CO92 (pgm–) for increasing time periods up to 14 days and under temperature conditions ranging from 4 °C (refrigeration) to 50 °C (desert conditions). DNA from each sample was extracted using the MasterPure Gram-positive DNA purification kit (Epicentre Biotechnologies [an Illumina company]; Madison, WI) and evaluated for three different criteria: (1) resultant DNA quality, (2) percent DNA recovery, and (3) effectiveness of recovered DNA in PCR analysis. Additional studies were performed to evaluate the ability of the method to be used to preserve viable bacteria after 1 or 2 days of storage at either 4 or 25 °C. These tests were also performed in an ISO/IEC 17025-compliant laboratory. The results for each of the preservation methods are reported in Section 5.

## 2. SELECTION OF CANDIDATE SYSTEMS (SAMPLE PREPARATIONS)

In the initial sample-preparation study, 20 commercially available nucleic acid isolation assays were evaluated for their effectiveness for use in DNA isolation for both a Gram-positive (*B. atrophaeus* var. *globigii*) and a Gram-negative organism (*Y. pestis* CO92 [pgm–] vegetative cells). Descriptions of the individual DNA preparation assays are shown in Table 1 and explained in detail below.

**Table 1. Manufacturer and Extraction Kit**

| List Manufacturer | DNA Extraction Kit |
|---|---|
| Akonni Biosystems, Inc. (Frederick, MD) | TruTip Microbial DNA |
| AutoGen, Inc. (Holliston, MA) | QuickGene-Mini80 DNA Purification System |
| Beckman Coulter, Inc. (Brea, CA) | Agencourt Genfind v2 DNA |
| bioMerieux, Inc. (Durham, NC) | NucliSens miniMAG |
| Bio-Rad Laboratories (Hercules, CA) | InstaGene Matrix |
| Claremont BioSolutions, LLC (Upland, CA) | PureLyse gDNA Extraction Kit |
| CUBRC, Inc. (Buffalo, NY) | DNAPro Extraction Pipette |
| Epicentre Biotechnologies (an Illumina Company; Madison, WI) | MasterPure Gram-Positive DNA Purification Kit |
| | SoilMaster DNA Extraction Kit |
| BioFire Diagnostics, Inc. (Salt Lake City, UT) | IT 1-2-3 Platinum Path Sample-Purification Kit |
| MoBio Laboratories, Inc. (Carlsbad, CA) | UltraClean Microbial DNA Isolation Kit |
| | PowerSoil DNA Isolation Kit |
| Molzym Life Sciences (Bremen, Germany) | PrestoSpin D Bug |
| MP Biomedicals, LLC (Santa Ana, CA) | FastDNA Spin Kit |
| | FastDNA Spin Kit for Soil |
| Promega Corporation (Madison, WI) | Maxwell 16 Forensic Instrument System |
| | Wizard Genomic DNA Purification Kit |
| Qiagen, Inc. (Valencia, CA) | QIAamp Stool Mini Kit |
| | DNeasy Blood and Tissue Kit |
| QuickSilver Analytics, Inc. (Abingdon, MD) | Lincoln Nucleic-Acid Kit (LiNK) 2.1 Complete Kit |

**Sample-Preparation Assays**

The following sample-preparation assays were evaluated during this study:

1. ***Akonni TruTip Microbial DNA***; *Akonni Biosystems, Inc.*: Akonni Biosystem's TruTip microbial DNA kit was a filter-based DNA purification system. The DNA-binding filter was located inside the specialized pipette tip. Liquid was aspirated into the tip and flowed back and forth across the filter matrix to allow for DNA binding. Wash and elution buffers were aspirated within the same pipette tip. To increase the output of this system, this procedure could be performed using a multichannel pipette. The final volume of eluted DNA was 75 µL.

2. ***Agencourt Genfind v2 DNA***; *Beckman Coulter, Inc.*: Beckman Coulter's Agencourt Genfind v2 DNA purification kit was used for extracting genomic DNA from several different sources including, but not limited to, whole blood, cultured eukaryotic cells, and bacteria. The DNA collection was based on solid-phase reversible immobilization paramagnetic beads. The cells were initially lysed with proteinase K and then bound to the paramagnetic beads. The beads were washed, and the DNA was eluted in tris-ethylenediaminetetraacetic acid (TE) buffer. This procedure did not require the use of phenol or chloroform extraction. The final elution amount was 100 µL.

3. ***bioMerieux NucliSens miniMAG***; *bioMerieux, Inc.*: bioMerieux's NucliSens miniMAG was an automated nucleic assay extraction system with magnetic silica beads to purify DNA from lysed samples. There were no requirements for ethanol or other organic solvents for this nucleic acid purification procedure. The miniMAG instrument was capable of performing 12 extractions in 60 min and the eluates collected from this process were immediately ready for downstream applications, such as PCR. The final elution volume was 50 µL.

4. ***Bio-Rad InstaGene Matrix***; *Bio-Rad Laboratories*: Bio-Rad's InstaGene Matrix was a Chelex resin-based, DNA purification assay. This system allowed for the purification of multiple types of samples including whole blood, eukaryotic cells, and bacteria. In this assay, cellular lysis involved boiling the samples in the presence of the matrix. There were no requirements for the use of phenol–chloroform or alcohol extractions. The final elution volume for this assay was 200 µL.

5. ***Claremont BioSolutions PureLyse gDNA Extraction Kit***; *Claremont BioSolutions*: Claremont's PureLyse gDNA extraction kit was designed to rapidly extract DNA from bacterial samples. In this assay, bacterial cells were suspended in binding buffer and aspirated several times through an OmniLyse cartridge that was attached to an external battery pack. The bacterial DNA bound to the cartridge was then eluted with elution buffer and dispensed into a new collection tube. The final volume of eluted DNA was 200 µL.

6. ***CUBRC DNAPro Extraction Pipette***; *CUBRC, Inc.*: CUBRC's DNA extraction pipette was a single-use tool that was based on the principles of solid-phase extraction, which was intended to sequentially isolate the nucleic acid and protein content of a sample in austere environments. The solid-phase extraction chemistries utilized for the extraction processes provided versatility in sample type and a broad range of user-defined modifications and adaptations. At the time of this report, this system was successfully tested with laboratory, water, and sewage sample matrices. The final elution volume was 300 µL.

7. ***Epicentre MasterPure Gram-Positive DNA Purification Kit***; *Epicentre Biotechnologies*: Epicentre's MasterPure Gram-positive DNA purification kit was specially designed for the collection of genomic DNA from Gram-positive bacteria. When using this protocol, bacteria were pretreated with Ready-Lyse lysozyme, followed by incubation in the Gram-positive cell-lysis solution. The residual protein was removed by treatment with the MasterPure Complete protein precipitation reagent, followed by alcohol-induced DNA precipitation. The resultant DNA pellet was resuspended in 35 µL of TE buffer.

8. ***Epicentre SoilMaster DNA Extraction Kit***; *Epicentre Biotechnologies*: Epicentre's SoilMaster DNA extraction kit was developed to isolate PCR-quality DNA from various types of soil samples. For this extraction kit, up to 100 mg of soil was weighed and placed into a 1.5 mL microcentrifuge tube. The soil was mixed with soil extraction buffer containing Proteinase K and incubated at 65 °C for 10 min. The samples were then centrifuged, supernatants were collected, and the DNA was precipitated by alcohol. The precipitated DNA was pelleted and resuspended in 35 µL TE buffer.

9. ***IT 1-2-3 Platinum Path Sample-Purification Kit***; *BioFire Diagnostics, Inc.*: BioFire Diagnostic's IT 1-2-3 Platinum Path sample-purification kit was a magnetic bead-based nucleic acid purification procedure that was used for purifying DNA from a wide variety of samples (e.g., eukaryotic cells, bacteria). For bacteria, the cells were lysed, and the DNA was extracted through bead beating. After this procedure, the DNA was bound to the magnetic beads and washed to remove any inhibitors. Finally, the DNA was eluted from the beads with 150 µL of water.

10. ***MoBio UltraClean Microbial DNA Isolation Kit***; *MoBio Laboratories, Inc.*: MoBio's UltraClean microbial DNA isolation kit was used for the purification of genomic DNA from Gram-negative and Gram-positive bacteria as well as eukaryotes such as yeast and fungi. It used bead beating followed by silica-based spin columns for purification. To use this kit, bacterial samples were mixed with a bead solution and then placed in bead-beating tubes containing beads and lysis solution. The cells were lysed using a combination of heat, detergent, and mechanical action. The released DNA was then bound to a silica filter in a spin column. The DNA was eluted in 50 µL of DNA-free Tris buffer.

11. ***MoBio PowerSoil DNA Isolation Kit***; *MoBio Laboratories, Inc.*: MoBio's PowerSoil DNA isolation kit was used for the purification of genomic DNA from organisms found in various types of soil. Up to 250 mg of soil was added to the PowerBead tubes that were included in the isolation kit, and then the tubes were vortexed. The samples were then centrifuged and the supernatant was collected. The resultant supernatants were placed in bead-

beating tubes containing beads and lysis solution. The cells were lysed using a combination of heat, detergent, and mechanical action. The released DNA was then bound to a silica filter in a spin column. The DNA was eluted in 50 µL of DNA-free Tris buffer.

12. ***Molzym PrestoSpin D Bug***; *Molzym Life Sciences*: Molzym Life Science's PrestoSpin D Bug was a clay, mineral-based filter, DNA purification kit for use with Gram-negative and Gram-positive bacteria. RNA could be removed from the system by adding RNase directly to the spin column. The bacterial cells were lysed with lysozyme, washed with buffer, loaded onto a spin column, and centrifuged. The samples were then washed, and the DNA was eluted with 75 µL of water.

13. ***MP FastDNA Spin Kit***; *MP Biomedicals, LLC*: MP Biomedicals' FastDNA spin kit was designed to be used for the purification of genomic DNA from plant and animal tissue, cultured cells, yeast, fungi, and bacteria. For bacteria, the cells were pelleted, resuspended in cell lysis solution, and then homogenized using MP Biomedical's FastPrep instrument. This homogenized solution was then centrifuged, and the supernatant was removed. The supernatant was mixed with an equal volume of binding matrix, agitated, and then transferred to a spin filter, and centrifuged. Finally, the DNA was eluted from the filter with 100 µL water.

14. ***MP FastDNA Spin Kit for Soil***; *MP Biomedicals, LLC*: MP Biomedical's FastDNA spin kit for soil was designed to be used for the purification of genomic DNA found in various soil samples. To use this kit, a soil sample no larger than 500 mg was mixed with specialized lysis solution and then homogenized using MP Biomedical's FastPrep instrument. This solution was then centrifuged, and the supernatant was collected. The supernatant was mixed with an equal volume of binding matrix and agitated. This solution was transferred to a spin filter and centrifuged, and the DNA was eluted with 100 µL water.

15. ***Promega Maxwell 16 Forensic Instrument System***; *Promega Corporation*: Promega's Maxwell 16 forensic instrument system was an automated nucleic acid extraction device that relied on paramagnetic beads that were used as a medium to bind and wash nucleic acids as part of the purification process. Several purification methods were preprogrammed into the instrument, and all of the necessary reagents were supplied as prefilled cartridges. It was capable of being used to process up to 16 samples in 30 min. The DNA recovered within the instrument was immediately ready for further processing (e.g., PCR analysis). The final elution volume was 100 µL.

16. ***Promega Wizard Genomic DNA Purification Kit***; *Promega Corporation*: Promega's Wizard genomic DNA purification kit was designed to extract DNA from several different sources including whole blood, animal tissue, cultured cells, yeast, bacteria, and plant tissue. The manufacturer developed specific protocols for Gram-positive and Gram-negative bacteria that were used for this study. To use this kit, the bacterial cultures were first centrifuged and pelleted. After this step, Gram-positive bacteria were treated with an additional lytic enzyme to weaken the cell walls before the cellular lysis step. The cells were lysed with Nuclei Lysis solution for 5 min at 80 °C. RNAs and proteins were removed through the addition of RNase and protein precipitation solutions. The mixtures were centrifuged, and the supernatants were

collected. The DNA was then precipitated by adding isopropanol. After the precipitation step, the isopropanol was removed, and the DNA was dried. Finally, the DNA was rehydrated by adding 100 µL of DNA rehydration solution and incubating it at 65 °C for 1 h.

17. ***QIAamp DNA Stool Mini Kit***; *Qiagen, Inc.*: Qiagen's QIAamp DNA Mini stool kit was designed for the purification of total DNA from fresh or frozen stool samples. There were no requirements for phenol–chloroform extraction or alcohol precipitation. To use this kit, the stool samples were first lysed in the provided lysis buffer at 70 °C to purify the bacterial DNA. This was followed by the inactivation of DNA-damaging substances and PCR inhibitors. Once this was complete, the DNA was purified using the standard protocol for QIAamp spin columns (see Qiagen DNeasy Blood & Tissue Kit, no. 18 in this list).

18. ***Qiagen DNeasy Blood and Tissue Kit***; *Qiagen, Inc.*: Qiagen's DNeasy blood and tissue kit was a silica-based DNA purification assay. It was a flexible system that could be used to purify DNA from many different cellular sources including human or animal tissue, eukaryotic cells, blood, or bacteria. There were no requirements for the use of phenol–chloroform extraction or alcohol precipitation. The initial purification step required the use of proteinase K for cellular lysis. Once the cells were lysed, the samples were loaded onto a spin column to remove any contaminants. The samples were then washed to remove any residual contaminates and eluted in water or buffer. The final elution amount for this assay was 200 µL.

19. ***QuickGene-Mini80 DNA Purification System***; *AutoGen, Inc.*: AutoGen's QuickGene-Mini80 DNA purification system was a filter-based process that required no centrifugation. It was a light, flexible system that allowed for the purification of both DNA and RNA from a variety of sample types. After an initial cell lysis step, the samples were mixed with ethanol, loaded onto a column, and placed in the purification apparatus. The samples were washed, and the nucleic acids were then eluted with 50 µL of water or TE buffer.

20. ***QuickSilver Lincoln Nucleic Acid Kit (LiNK) 2.1 Complete Kit***; *QuickSilver Analytics, Inc.*: QuickSilver Analytics's LiNKs 2.1 was a disposable, no-power cartridge that was used to collect and purify environmental samples in the field, which yielded a DNA-containing eluate (1000 µL) that was compatible with PCR machines.

## 3. LABORATORY ASSESSMENT (SAMPLE PREPARATION)

### 3.1 Sample-Preparation Study Materials and Methods

The laboratory assessment portion of this study included specific materials and procedures for sample preparation that are described in Sections 3.1.1 through 3.1.6.

### 3.1.1 Reference Materials

For the current study, Gram-positive and Gram-negative organisms were used to examine the effectiveness of several commercially available nucleic acid purification assays. For the Gram-positive organism, *B. atrophaeus* var. *globigii* (Unified Culture Collection [UCC] designation: BACI051) was selected as a surrogate for the well-known biothreat agent *Bacillus anthracis*. For the Gram-negative organism, *Y. pestis* CO92 (pgm–) (UCC designation: YERS059) was selected. Both bacterial stocks used in this study were obtained from the Critical Reagents Program (Frederick, MD).

### 3.1.2 Nucleic Acid Extraction

Twenty different commercially available DNA purification assays were evaluated in this study; the names of the extraction kits and the kit manufacturers are listed in Table 1. For each assay, the DNA purifications were performed according to the manufacturers' recommended protocols. DNA purification was performed on viable cell concentrations of $10^7$ or $10^9$ cfu/sample for each agent tested. Additionally, 10 µg of isolated DNA, from each type of bacteria examined, was used to determine the relative efficiency of each DNA purification assay. All laboratory procedures were performed in the ECBC BioSensors Branch laboratories under ISO/IEC 17025 compliance (ISO, 2005).

### 3.1.3 Qualitative Nucleic Acid Determination

DNA extracted from either *B. atrophaeus* spores or *Y. pestis* CO92 (pgm–) vegetative cells was analyzed for purity using the ThermoScientific NanoDrop spectrophotometer model 2000c (Pittsburgh, PA). DNA absorbance for each sample was evaluated in the elution buffers provided with each DNA extraction assay; the NanoDrop instrument was blanked prior to each measurement using the elution buffers specific to each isolation assay. Absorbance measurements at 260 nm ($A_{260}$) and 280 nm ($A_{280}$) were taken for each sample. To determine the purity for the collected DNA, the ratio between $A_{260}$ and $A_{280}$ was calculated. An absorbance ratio ($A_{260}/A_{280}$) of 1.8/2.0 was considered to be contaminant-free DNA. Results were reported as the mean ± standard error of the mean (SEM) of resultant $A_{260}/A_{280}$ ratios.

### 3.1.4 Quantitative Nucleic Acid Determination

To evaluate the amount of DNA recovered for each sample, the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies; Grand Island, NY) was used according to the manufacturer's suggested protocol. Three individual samples from each DNA purification assay were used; each of these individual samples was examined in triplicate. The results for each sample were reported as nanograms per milliliter and expressed as percent mean recovery of total initial DNA ± SEM.

### 3.1.5 Real Time (RT)-PCR Evaluation of Purified Nucleic Acid

Extracted DNA samples were analyzed for PCR amplification on an ABI 7900HT sequence-detection system (Life Technologies). Experimental samples were tested at a concentration of 0.02 ng/µL, which was made by adding 5 µL of the DNA sample to 15 µL of master mix. The samples were then cycled in two stages. Stage 1 consisted of one cycle at 50 °C for 2 min and one cycle at 95 °C for 20 s. Stage 2 consisted of 45 cycles at 95 °C for 1 s, followed by 60 °C for 20 s. The 6-carboxyfluorescein and tetramethylrhodamine (FAM/TAMRA)-labeled primers for BACI051 and YERS059 were obtained through the Critical Reagents Program and used as directed. RT-PCR results for BACI051 and YERS059 were considered positive if the cycle threshold (Ct) value was <40. Negative results were considered to be Ct values of >40 or absent/undetermined Ct values. Results were reported as mean ± SEM of resultant Ct values.

### 3.1.6 Evaluation of DNA Extraction Assays Using Different Soil Matrices

To determine the effects of preparing samples from different matrices, *B. atrophaeus* var. *globigii* spores were spiked in standardized reference clay and sand samples as described by the National Institute of Standards and Technology. The soil samples used in this study were provided by Dr. Ron Checkai, Chief of Environmental Toxicology at ECBC. For these studies, the following six DNA extraction kits were evaluated:

- InstaGene Matrix from Bio-Rad;
- SoilMaster DNA extraction kit from Epicentre Biotechnologies;
- IT 1-2-3 Platinum Path sample-purification kit from BioFire Diagnostics, Inc.;
- PowerSoil DNA isolation kit from MoBio Laboratories, Inc.;
- FastDNA spin kit for soil from MP Biomedicals, LLC; and
- QIAamp DNA stool mini kit from Qiagen, Inc.

Soil results were analyzed for DNA purity and by RT-PCR as described in Section 3.1.5. DNA purity values were reported as mean ± SEM of resultant $A_{260}/A_{280}$ ratios and PCR results were reported as mean ± SEM of resultant Ct values.

## 3.2 Sample-Preparation Study Results

Table 2 shows a comparison of DNA extraction kits with respect to the cost per sample, processing time, ability to process multiple samples at once, and whether or not additional equipment was required.

**Table 2. Comparison of Nucleic Acid Extraction Kits**

| Nucleic Acid Extraction Kit | Cost/Sample ($) | Processing Time (min) | Maximum No. Samples | Additional Required Equipment |
|---|---|---|---|---|
| Akonni TruTip | 5.17 | 15–60 | 8 | Rainin pipette, thermomixer |
| Beckman Coulter Agencourt Genfind | 9.70 | 80 | 10 | Magnet stand |
| bioMerieux NucliSens miniMAG | 5.50 | 35 | 12 | miniMag device |
| Bio-Rad InstaGene Matrix | 0.71 | 30–45 | 22 | N/A |
| Claremont PureLyse | 7.50 | 5 | 1 | N/A |
| CUBRC DNAPro | 1.00 | 20 | 1 | N/A |
| Epicentre MasterPure | 2.55 | 150 | 22 | N/A |
| Epicentre SoilMaster | 4.40 | 50 | 22 | N/A |
| BioFire Diagnostics IT 1-2-3 Platinum Path | 12.70 | 15 | 1 | Magnetic pick pen |
| MoBio UltraClean | 2.74 | 45 | 22 | Vortex adaptor |
| MoBio PowerSoil | 4.98 | 40 | 22 | Vortex adaptor |
| Molzym PrestoSpin D Bug | 3.70 | 30–50 | 22 | N/A |
| MP FastDNA Spin Kit | 3.75 | 20–25 | 22 | FastPrep instrument |
| MP FastDNA Spin Kit for Soil | 5.30 | 30–35 | 22 | FastPrep instrument |
| Promega Maxwell 16 | 2.97 | 45–180 | 8 | Maxwell 16 instrument |
| Promega Wizard | 1.80 | 155–225 | 22 | N/A |
| Qiagen QIAamp Stool Mini Kit | 4.30 | 35–40 | 22 | N/A |
| Qiagen DNEasy Blood and Tissue Kit | 3.30 | 60–90 | 22 | N/A |
| AutoGen QuickGene-Mini80 | 0.30 | 60–90 | 8 | QuickGene instrument, AutoGen reagents |
| QuickSilver LiNK 2.1 | 59.00 | 15 | 1 | N/A |

N/A: not applicable

Evaluating a cost analysis of the 20 DNA preparation kits (Table 2) revealed that the QuickGene-Mini80 DNA purification system had the lowest cost per sample ($0.30), but did require the initial purchase of the QuickGene apparatus ($1,825.00) and AutoGen reagents. Bio-Rad's Instagene Matrix had an estimated cost per sample of $0.71 and did not require any additional equipment to run. QuickSilver's LiNK 2.1 complete kit had the highest cost per sample at $59.00. Use of the Claremont PureLyse gDNA extraction kit produced the fastest processing time for the assays examined in this study at 5 min; however, this assay was only

capable of preparing a single sample at a time. If the amount of samples that can be processed at once are taken into account in the average processing time, the Bio-Rad Instagene Matrix, the MP FastDNA Spin kit, and the MP FastDNA Spin kit for soil were each capable of processing 22 samples in approximately 30–40 min. Using the Promega Wizard Genomic DNA purification kit produced the longest processing time of approximately 2.5–3 h. Table 3 shows a comparison of the average purity of DNA extraction performed using DNA extraction kits on *B. atrophaeus* var. *globigii* and *Y. pestis* CO92 (pgm–) vegetative cells.

**Table 3. Comparison of Average Purity of DNA**

| Nucleic Acid Extraction Kit | $A_{260}/A_{280}$ | | | | | |
|---|---|---|---|---|---|---|
| | ***B. atrophaeus* var. *globigii*** | | | ***Y. pestis* CO92 (pgm–) vegetative cells** | | |
| | **DNA** | **$10^7$** | **$10^9$** | **DNA** | **$10^7$** | **$10^9$** |
| Akonni TruTip | 1.65 ± 0.17 | 1.32 ± 0.06 | 1.33 ± 0.03 | 1.81 ± 0.01 | 1.46 ± 0.03 | 1.82 ± 0.03 |
| Beckman Coulter Agencourt Genfind | 1.86 ± 0.04 | 1.66 ± 1.13 | 2.19 ± 0.27 | 1.84 ± 0.00 | 2.35 ± 0.14 | 2.09 ± 0.00 |
| bioMerieux NucliSens miniMAG | 1.54 ± 0.04 | 0.91 ± 0.04 | 1.49 ± 0.14 | 2.25 ± 0.06 | 0.17 ± 0.05 | 2.30 ± 0.03 |
| Bio-Rad InstaGene Matrix | 2.09 ± 0.01 | 1.12 ± 0.24 | 1.28 ± 0.01 | 1.67 ± 0.09 | 1.34 ±0.01 | 1.65 ± 0.01 |
| Claremont PureLyse | 1.72 ± 0.01 | 1.81 ± 0.01 | 2.75 ± 0.07 | 1.66 ± 0.02 | 1.27 ± 0.01 | 1.67 ± 0.01 |
| CUBRC DNAPro | 1.65 ± 0.17 | 1.19 ± 0.07 | 3.30 ± 0.75 | 6.23 ± 4.60 | 2.48 ± 0.28 | 2.36 ± 0.06 |
| Epicentre MasterPure | 1.88 ± 0.01 | 2.05 ± 0.22 | 1.94 ± 0.06 | 1.91 ± 0.01 | 1.96 ± 0.06 | 1.98 ± 0.00 |
| Epicentre SoilMaster | 1.86 ± 0.05 | 1.64 ± 0.04 | 1.88 ± 0.10 | 1.71 ± 0.23 | 3.02 ± 1.67 | 1.32 ± 2.62 |
| BioFire IT 1-2-3 Platinum Path | 1.63 ± 0.16 | 1.06 ± 0.80 | 1.70 ± 0.08 | 1.90 ± 0.08 | 1.52 ± 0.05 | 1.71 ± 0.04 |
| MoBio UltraClean | 1.91 ± 0.05 | 1.25 ± 0.01 | 1.58 ± 0.03 | 1.85 ± 0.01 | 1.53 ± 0.01 | 1.86 ± 0.01 |
| MoBio PowerSoil | 2.31 ± 0.19 | 0.80 ± 0.45 | 0.02 ± 0.93 | 1.76 ± 0.02 | 1.44 ± 0.08 | 1.76 ± 0.01 |
| Molzym PrestoSpin D Bug | 1.72 ± 0.01 | 1.81 ± 0.01 | 2.75 ± 0.07 | 1.59 ± 0.07 | 1.66 ± 0.04 | 2.05 ± 0.03 |
| MP FastDNA Spin Kit | 1.19 ± 0.03 | 1.27 ± 0.01 | 1.24 ± 0.01 | 1.75 ± 0.01 | 1.52 ± 0.01 | 1.69 ± 0.01 |
| MP FastDNA Spin Kit for Soil | 1.86 ± 0.06 | 2.29 ± 0.11 | 2.06 ± 0.10 | 1.91 ± 0.01 | 1.77 ± 0.03 | 1.86 ± 0.00 |
| Promega Maxwell 16 | 1.70 ± 0.15 | 0.69 ± 0.13 | 1.00 ± 0.04 | Not done | 0.36 ± 0.09 | 2.02 ± 0.02 |
| Promega Wizard | 1.83 ± 0.01 | 0.57 ± 0.80 | 1.54 ± 0.03 | 2.09 ± 0.01 | 1.27 ± 0.01 | 1.96 ± 0.05 |
| Qiagen QIAamp Stool Mini Kit | 1.65 ± 0.09 | 1.53 ±0.12 | 1.63 ± 0.13 | 2.08 ± 0.03 | Undetermined | 2.11 ± 0.02 |
| Qiagen DNEasy Blood and Tissue Kit | 1.86 ± 0.01 | 0.85 ± 0.15 | 2.76 ± 0.51 | 1.80 ± 0.00 | 1.54 ± 0.02 | 1.88 ± 0.04 |
| QuickGene-Mini80 | 1.87 ± 0.02 | 1.58 ± 1.84 | 1.93 ± 0.04 | 2.05 ± 0.03 | Undetermined | 1.81 ± 0.01 |
| QuickSilver LiNK 2.1 | 3.38 ± 0.09 | 2.64 ± 0.09 | 2.05 ± 0.05 | 3.84 ± 0.19 | 1.28 ± 0.01 | 1.26 ± 0.01 |

Several nucleic acid isolation kits were used to produce pure DNA after processing either *B. atrophaeus* or *Y. pestis* from the previously isolated control DNA (Table 3). The list of successful purifications was reduced when DNA was purified from either *B. atrophaeus* spores or *Y. pestis* vegetative cells. The Claremont PureLyse gDNA and Epicentre SoilMaster DNA extraction kits, the Molzym PrestoSpin D Bug, and the QuickGene-Mini80 DNA purification system were used to produce purified *B. atrophaeus* DNA (from $10^7$ or $10^9$ cells) within an $A_{260}/A_{280}$ ratio of 1.8/2.0. Several kits, but not all of those tested, produced DNA that was isolated from *Y. pestis* vegetative cells ($10^7$ or $10^9$) with $A_{260}/A_{280}$ ratios of 1.8/2.0. These kits included the Akonni Microbial DNA kit, Epicentre MasterPure Gram-Positive DNA purification kit, MoBio UltraClean Microbial DNA isolation kit, MP FastDNA Spin kit for soil, Promega Wizard Genomic DNA purification kit, Qiagen DNeasy blood and tissue kit, and the QuickGene-Mini80 DNA purification system.

For the *B. atrophaeus* samples, there was little consistency among the nucleic acid preparation kits (Figures 1A, C, and E). When used to process *B. atrophaeus* DNA, Qiagen's DNeasy kit produced the best performance with approximately 55% DNA recovery. The QuickGene-Mini80 DNA purification system was used to recover approximately 30% of the possible DNA within the $10^7$ *B. atrophaeus* spores. The QuickSilver LiNK 2.1 kit was used to recover approximately 15% of the DNA within $10^9$ *B. atrophaeus* spores. When compared with the *B. atrophaeus* samples, there was generally more consistency in performance and greater recovery with the *Y. pestis* samples (Figures 1B, D, and F). When *Y. pestis* DNA recovery was examined, Promega's Maxwell 16 and Qiagen's DNeasy kits were used to recover ≥75% of the originally loaded DNA. In the $10^7$ samples, Agencourt's Genfind v2 DNA and Qiagen's DNeasy kits were used to recover ≥75% of the DNA associated with this many bacterial cells. For the *Y. pestis* $10^9$ samples, four kits had ≥40% recovery; these kits were the Qiagen DNeasy and Agencourt Genfind v2 DNA kits, the MoBio PowerSoil DNA isolation kit, and the MP FastDNA Spin kit for soil.

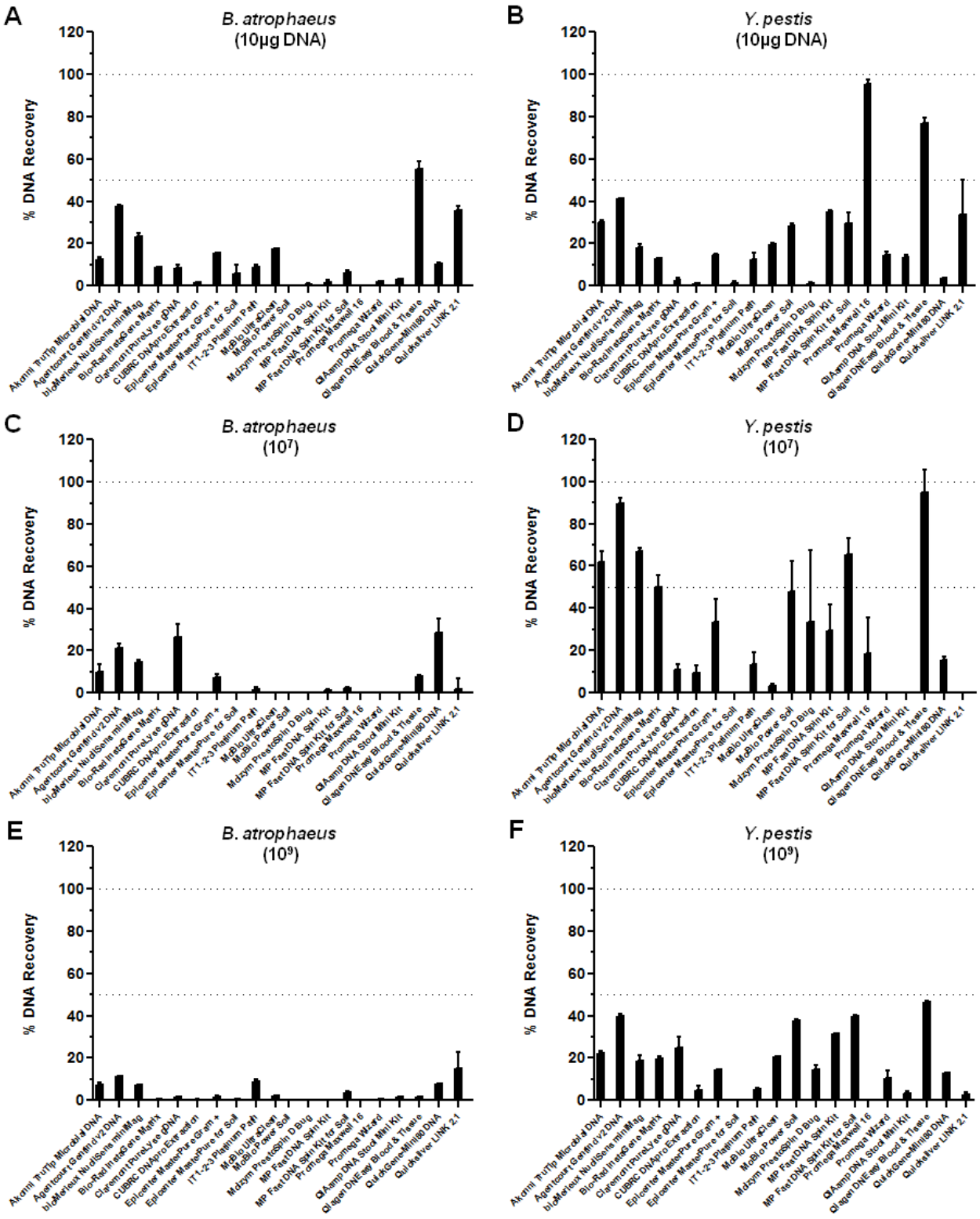


**Figure 1. Comparison of collection efficiency for total DNA recovery using DNA extraction kits on *B. atrophaeus* var. *globigii* and *Y. pestis* CO92 (pgm–) vegetative cells.**

Almost all of the kits tested were able to be used to isolate DNA that provided positive-PCR results (Table 4). For the *B. atrophaeus* studies, Promega's Wizard Genomic DNA purification kit failed to produce positive PCR results at both $10^7$ and $10^9$ cfu. Use of the QuickGene-Mini80 DNA purification system did not produce any results following the processing of previously isolated *B. atrophaeus* control DNA. In the *Y. pestis* studies, using the MP FastDNA Spin kit failed to produce results for all three conditions.

**Table 4. Comparison of DNA Extraction Kits on the Basis of the Average Ct Values**

| Nucleic Acid Extraction Kit | Ct Values | | | | | |
|---|---|---|---|---|---|---|
| | ***B. atrophaeus* var. *globigii*** | | | ***Y. pestis* CO92 (pgm–) vegetative cells** | | |
| | **DNA** | **$10^7$** | **$10^9$** | **DNA** | **$10^7$** | **$10^9$** |
| Akonni TruTip | 15.85 ± 0.21 | 24.72 ± 1.86 | 16.59 ± 0.02 | 10.18 ± 0.10 | 17.69 ± 0.18 | 12.48 ± 0.29 |
| Beckman Coulter Agencourt Genfind | 27.33 ± 1.46 | 27.68 ± 0.42 | 25.60 ± 1.38 | 11.08 | 17.72 ± 0.03 | 11.73 ± 0.03 |
| bioMerieux NucliSens miniMAG | 14.79 ± 0.12 | 22.38 ± 0.09 | 16.85 ± 0.05 | 13.21 ± 0.04 | 17.89 ± 0.02 | 13.64 ± 0.25 |
| Bio-Rad InstaGene Matrix | 23.42 ± 0.34 | 25.79 ± 0.03 | 23.84 ± 0.25 | 11.96 | 18.28 ± 0.11 | 11.98 ± 0.06 |
| Claremont PureLyse | 23.62 ± 0.06 | 24.90 ± 0.21 | 24.12 ± 0.05 | 17.85 ± 0.62 | 22.25 ± 0.07 | 14.83 ± 0.04 |
| CUBRC DNAPro | 20.45 ± 0.16 | 29.10 ± 0.52 | 23.95 ± 0.81 | 19.54 ± 0.51 | 22.50 ± 0.25 | 17.83 ± 0.69 |
| Epicentre MasterPure | 26.51 | 25.97 ± 1.33 | 23.44 ± 0.04 | 12.53 | 18.09 ± 0.55 | 14.08 |
| Epicentre SoilMaster | 25.55 | 27.84 ± 0.27 | 29.18 ± 2.77 | 15.26 ± 2.81 | 28.97 ± 0.66 | 22.30 ± 0.78 |
| BioFire IT 1-2-3 Platinum Path | 23.59 ± 0.08 | 26.31 ± 0.13 | 23.25 ± 0.16 | 14.83 ± 0.20 | 19.90 ± 0.32 | 15.87 ± 0.09 |
| MoBio UltraClean | 14.60 ± 0.12 | 31.46 ± 1.17 | 20.09 ± 1.09 | 11.13 ± 0.07 | 25.13 ± 0.44 | 10.55 ± 0.08 |
| MoBio PowerSoil | 21.90 ± 3.34 | 31.58 ± 2.53 | 20.64 ± 1.25 | 12.34 ± 0.10 | 18.84 ± 0.08 | 11.84 ± 0.02 |
| Molzym PrestoSpin D Bug | 23.67 ± 0.38 | 35.83 ± 1.74 | 25.79 ± 0.41 | 15.63 | 21.65 ± 2.49 | 14.25 ± 0.47 |
| MP FastDNA Spin Kit | 18.35 ± 0.59 | 30.52 ± 0.41 | 22.54 ± 1.74 | Undetermined | Undetermined | Undetermined |
| MP FastDNA Spin Kit for Soil | 18.07 ± 0.19 | 29.68 ± 0.53 | 21.15 ± 0.27 | 12.07 ± 0.44 | 17.91 ± 0.17 | 11.17 ± 0.22 |
| Promega Maxwell 16 | 23.35 ± 0.09 | 33.34 ± 0.38 | 23.80 ± 0.06 | 11.52 ± 0.08 | 18.20 ± 0.06 | 11.51 ± 0.04 |
| Promega Wizard | 23.81 | 44.51 ± 0.44 | Undetermined | 13.36 ± 0.30 | 23.72 ± 0.14 | 14.82 ± 0.62 |
| Qiagen QIAamp Stool Mini Kit | 23.05 ± 0.04 | 27.03 ± 0.14 | 23.47 ± 0.04 | 13.43 ± 0.21 | 23.45 ± 0.26 | 17.45 ± 0.28 |
| Qiagen DNEasy Blood and Tissue Kit | 15.06 ± 0.16 | 24.29 ± 0.15 | 20.20 ± 0.14 | 11.47 ± 0.13 | 18.74 ± 0.13 | 13.74 ± 0.07 |
| QuickGene-Mini80 | Undetermined | 23.68 ± 0.17 | 23.95 ± 0.19 | 14.74 ± 0.05 | 19.61 ± 0.13 | 15.27 ± 0.12 |
| QuickSilver LiNK 2.1 | 18.14 ± 0.49 | 30.02 ± 3.12 | 23.34 ± 0.26 | 14.31 ± 0.10 | 24.64 ± 0.08 | 19.21 ± 0.36 |

Only Epicentre's SoilMaster DNA extraction kit was used to produce acceptable $A_{260}/A_{280}$ results at $10^7$ *B. atrophaeus* spores in clay, but these data had a large SEM (Table 5).

**Table 5. Comparison of Average Purity of DNA Extraction Performed Using DNA Extraction Kits with *B. atrophaeus* var. *globigii* Spiked-Soil Samples**

| Nucleic Acid Extraction Kit | $A_{260}/A_{280}$ | | | |
|---|---|---|---|---|
| | Clay | | Sand | |
| | $10^7$ | $10^9$ | $10^7$ | $10^9$ |
| Bio-Rad InstaGene Matrix | 1.45 | 1.44 | 1.33 ± 0.01 | 1.35 |
| Epicentre SoilMaster | 1.90 ± 0.36 | 1.01 ± 0.29 | 0.75 ± 0.06 | 0.95 ± 0.03 |
| BioFire IT 1-2-3 Platinum Path | 1.35 | 1.35 | 1.35 | 1.35 |
| MoBio PowerSoil | 1.40 ± 0.02 | 1.40 ± 0.01 | 1.45 ± 0.03 | 1.61 ± 0.05 |
| MP FastDNA Spin Kit Soil | 1.44 ± 0.01 | 1.51 ± 0.01 | 1.34 ± 0.01 | 1.38 ± 0.01 |
| Qiagen QIAamp Stool Kit | 1.35 | 1.35 ± 0.01 | 1.35 ± 0.03 | 1.40 ± 0.01 |

Four of the six kits that were examined produced positive PCR results after the isolation of *B. atrophaeus* DNA from clay and/or sand (Table 6). The Epicenter SoilMaster DNA extraction, the IT 1-2-3 Platinum Path sample purification, and the MoBio PowerSoil DNA isolation kits produced positive PCR results using both matrices. Only the QIAamp stool kit produced positive PCR results when the sand matrix was used.

**Table 6. Comparison of DNA Extraction Kits on the Basis of Average Ct Values with *B. atrophaeus* var. *globigii* Spiked-Soil Samples**

| Nucleic Acid Extraction Kit | Ct Values | | | |
|---|---|---|---|---|
| | Clay | | Sand | |
| | $10^7$ | $10^9$ | $10^7$ | $10^9$ |
| Bio-Rad InstaGene Matrix | Undetermined | Undetermined | Undetermined | Undetermined |
| Epicentre SoilMaster | 26.74 ± 0.22 | 21.17 ± 0.21 | 26.99 ± 0.38 | 22.14 ± 0.17 |
| BioFire IT 1-2-3 Platinum Path | 32.49 ± 0.67 | 26.27 ± 0.84 | 33.13 ± 0.58 | 24.25 ± 0.22 |
| MoBio PowerSoil | 23.30 ± 0.08 | 17.86 ± 0.05 | 22.85 ± 0.04 | 17.42 ± 0.36 |
| MP FastDNA Spin Kit for Soil | Undetermined | Undetermined | Undetermined | Undetermined |
| Qiagen QIAamp Stool Mini Kit | Undetermined | Undetermined | 26.76 ± 0.05 | 23.55 ± 0.04 |

### 3.3 Sample-Preparation Study User Reviews

The following sample-preparation assays from various companies were evaluated and reviewed during this study:

1. ***Akonni TruTip Microbial DNA***: The Akonni TruTip microbial DNA kit employed a Rainin pipette to individually wash and extract DNA from a sample. In total, the protocol took 15 min per sample from start to finish. For the purpose of this study, nine samples of Gram-negative and Gram-positive organisms were extracted. One operator noted that using the Akonni kit would be much easier with a multichannel pipette, but completing each sample individually with a single-channel pipette was "highly time-consuming and repetitive." The three wash steps and multiple elution cycles performed well in purifying the Gram-negative samples, but results were less impressive with Gram-positive samples.

2. ***Agencourt Genfind v2 DNA***: The Agencourt Genfind v2 DNA extraction kit employed magnetic binding beads for DNA purification. The kit required 90 min to complete and ensured purified DNA with four wash steps. The kit required a magnet stand for separation, which was not included; therefore, the use of the magnet stand limited the number of samples that could be processed at one time. We were able to process 10 bacterial samples at once. The protocol allowed for samples only up to 400 µL; larger volume samples would require an additional concentrating step. It should be noted that a 96-well plate protocol was available but would have required a different magnet plate. A user commented, "The simplicity of use is great for singular- or multiple-sample processing."

3. ***bioMerieux NucliSens miniMAG***: The bioMerieux NucliSens miniMAG employed magnetic beads for DNA extraction. The total extraction time required 45 min to complete and purified DNA using five wash steps. The kit required the use of a thermomixer and a magnet stand, which are not included. These items were to be used with the NucliSens reagents and miniMAG. The miniMAG was an electronic magnet stand that made the washing of samples an easier process than its manual competitors. We were able to process 12 samples at one time, using sample sizes up to 1 mL. A reviewer stated, "While reagents must be kept refrigerated and the electric magnet can be bulky, the ease-of-use and simplistic protocol makes using the miniMAG very efficient."

4. ***Bio-Rad InstaGene Matrix***: The InstaGene Matrix was one of the fastest kits used in this study, with the ability to process up to 22 samples at a time in only 30–45 min, depending on the chosen incubation time. The protocol itself was remarkably simple and required very few materials, which made this kit useful for both large and small sample volumes. One scientist even went as far as to say, "I wish every kit was designed like the Bio-Rad Instagene Matrix." Although the quality of DNA did not qualify in the ideal range, the extracted samples performed well in PCR testing. The user-friendly protocol, combined with the low cost and time efficiency, made this kit one of the favorites among the scientists.

5. ***Claremont BioSolutions PureLyse gDNA Extraction Kit***: The Claremont BioSolutions PureLyse Kit was best used for the DNA extraction of small volume samples. This kit was unique in that it used battery-operated cartridges attached to syringes that filter samples

as a means of DNA isolation. Some advantages to this kit were that it required very few materials and was fairly quick, if used with small amounts of sample. However, this kit was ideally used only with a small quantity at one time because of the syringe method. For multiple samples or larger sample quantities, the kit became much more time-consuming and repetitive. In addition, users stated that attempting to hold the battery pack with the syringe while simultaneously trying to manage the sample proved to be difficult. Overall, users appreciated the concept of the PureLyse kit but found it to be problematic in practice.

6. ***CUBRC DNAPro Extraction Pipette***: The CUBRC nucleic acid and protein isolation kit washed and isolated DNA using a Pasteur pipette. Each sample was processed individually to fruition and took about 20 min to set up and complete. Because multiple samples could not be processed at once, using this kit became highly redundant and time-consuming after a few samples. A major problem encountered with the CUBRC kit was that a portion of the liquid repeatedly got stuck in the bulb of the pipette and could not be extracted. Consequently, the results yielded for both *B. atrophaeus* and *Y. pestis* samples were not up to specification. Despite a user-friendly protocol that included visual aids, users rated the CUBRC kit as an impractical means for DNA extraction beyond one or two samples.

7. ***Epicentre MasterPure Gram-Positive DNA Purification Kit***: The Epicentre MasterPure Gram-positive DNA purification kit utilized a DNA-precipitation method and had a sample-processing time around 2.5 h, with leniency on the incubation times. One advantage of this kit was that, unlike many other DNA extraction kits, using multiple samples at once did not have a drastic effect on the overall processing time, which made it a reliable choice regardless of the sample-processing quantity. For the use of our study, we found the option to incubate samples overnight beneficial. All samples were first pelleted in this protocol; therefore, sample volume size was not limited. Some of the required reagents needed to be kept frozen. The Gram-positive DNA purification kit was recommended by a user stating, "With such a thorough and easily understood protocol, and the required use of a lysozyme, the Epicentre MasterPure Gram-positive DNA purification kit is a great option for Gram-positive bacteria."

8. ***Epicentre SoilMaster DNA Extraction Kit***: The Epicentre SoilMaster DNA extraction kit was an effective kit that employed a precipitation method for processing soil samples. With a sample-processing time of approximately 50 min, an advantage of the Epicentre kit was that, unlike many other DNA extraction kits, it was designed for processing multiple samples at once. The soil sample size was limited to the centrifuge tube size. One user commented, "The protocol is easy to follow and includes spin columns, which are useful for purifying soil samples."

9. ***IT 1-2-3 Platinum Path Sample-Purification Kit***: The IT 1-2-3 Platinum Path sample-purification kit entailed a unique protocol that used a magnetic PickPen (not included with reagents) to transfer a wide range of sample types through various pre-aliquoted wash solutions for purification. When this kit was used for multiple samples, the first sample took 15 min to process, and each sample thereafter took an additional 2 min, which made this kit very efficient. In addition, the PickPen was surprisingly effective at transferring the sample to and from the various solutions. Because of its efficiency and ease-of-use, users agreed that they would recommend this kit.

10. ***MoBio UltraClean Microbial DNA Isolation Kit***: The MoBio UltraClean microbial DNA isolation kit employed a filter-based approach for DNA purification. With a 45 min processing time, the protocol used microbead tubes and spin filters to aid with lysis and filtration. Users had the option of using a detailed protocol that explains the purpose of each step or using a condensed, experienced user version of the protocol. This kit allowed for great ease-of-use when processing multiple samples at once. A user commented, "The UltraClean extraction kit makes processing numerous samples at one time very efficient, while not drastically increasing the overall time to process from start to finish."

11. ***MoBio PowerSoil DNA Isolation Kit***: The MoBio PowerSoil DNA isolation kit employed a silica filter binding method to extract and purify DNA and took just 40 min for soil sample processing. Similar to the MoBio UltraClean microbial DNA isolation kit, it included a concise, experienced user protocol and a more-detailed protocol to describe the purpose of each individual step in depth, so that the user could better understand the process. The protocol provided alternative lysis methods to potentially reduce sheering and specific instructions for wet soil samples. Also, MoBio made processing multiple samples very easy, while not increasing processing time. The soil sample size was limited to centrifuge tube size. One user commented, "My favorite part about the MoBio PowerSoil kit is that the protocol is similar to the MoBio UltraClean microbial DNA isolation kit."

12. ***Molzym PrestoSpin D Bug DNA Purification Kit***: The Molzym Prestospin D Bug DNA purification kit included a unique clay-mineral filter-binding system for extraction. Most of the supplies needed for the sample processing were included in the kit, which simplified preparation. The protocol was easy to follow and relatively short, with a total processing time of 30–50 min, depending on the chosen incubation period. Additionally, the protocol provided warnings regarding the buffers that were irritants to help ensure caution when using them. Because the kit employed spin filters for extraction, processing multiple samples at once was easy. A user stated, "Based on my experience, the Molzym PrestoSpin D is an excellent kit for DNA purification with its easy to read protocol and ability to process multiple or singular samples".

13. ***MP FastDNA Spin Kit***: The MP FastDNA spin kit was a quick-lysing method for DNA extraction using filters. With an easy-to-follow protocol, the extractions took approximately 25 min. Additionally, this kit was ideal for processing multiple samples at once without adding too much time to the extraction. Using this kit required a FastPrep instrument for the quick-lysing step. Larger sample volumes could be pelleted for use, which would allow the kit to be used to process large or small volume sample. Users agreed that they would recommend the MP FastDNA Spin Kit for extraction of large or small volume samples.

14. ***MP FastDNA Spin Kit for Soil***: The MP FastDNA spin kit for soil was a filter-based method of DNA extraction from multiple types of soil samples. The kit employed multiple wash solutions to ensure DNA purity. In addition, the spin filters included in the kit helped to expedite the sample-processing time to 35 min. Similar to the MP FastDNA spin kit, it could be easily used to process multiple samples at once without adding to the overall processing time. This kit required the use of a FastPrep instrument for lysing. Soil sample size was limited

to centrifuge tube size. Regarding the FastPrep instrument, one user commented that "the lysing step is very fast and efficient."

15. ***Promega Maxwell 16***: Promega's Maxwell 16 was an easy-to-use, fully-automated, sample-processing instrument. The Maxwell 16 instrument purified samples by using paramagnetic particles (PMPs) to optimize capture, washing, and elution of the target material. The instrument employed prefilled reagent cartridges and a magnetic-handling system to move the PMPs to each compartment of the cartridges. According to the NanoDrop data within our report, the Maxwell 16 was able to isolate DNA from both spores and vegetative cells, even in the presence of clay and sand soils.

16. ***Promega Wizard Genomic DNA Purification Kit***: The Promega Wizard genomic DNA purification kit was an easy-to-follow DNA-precipitation method for purification of various sample types. The method differentiated between the cell types with slightly different protocols. The Gram-negative bacteria culture took around 2 h to purify, whereas the Gram-positive bacterial culture took around 3 h. Although the kit was time-consuming to use, the visuals on the protocol gave a helpful overview of what to expect throughout the process. The kit did allow for processing multiple or singular samples. Low DNA yields could make the precipitation step difficult. Because of the long processing time, one user noted that this kit, "Would be most beneficial when using larger sample quantities, given that the processing time is not highly affected."

17. ***QIAamp DNA Stool Mini Kit***: The QIAamp DNA stool kit used filters for extraction of DNA from fresh or frozen stool samples. The kit took 40 min to complete. A significant advantage to this kit was that it was designed to process multiple samples at once, rather than processing samples individually from start to finish. This feature saved a great deal of processing time. Additionally, the spin columns that were included in the kit helped to further filter the DNA, and the multiple wash steps increased the DNA purity. The sample size was dependent on the centrifuge tube size. Users agreed that they would recommend this kit for DNA extraction.

18. ***Qiagen DNeasy Blood and Tissue Kit***: The Qiagen DNeasy blood and tissue kit employed filter columns to purify DNA from a variety of samples. The processing times were 1 and 1.5 h for Gram-negative and Gram-positive bacteria, respectively. The manufacturer's instructions were very easy to follow, and all of the required materials came with the kit. The spin columns made processing multiple samples at one time very efficient, and the wash steps enabled greater purity of the extracted DNA. The filters could become blocked if cell concentration was too high; therefore, a dilution could be necessary. Users stated that they would recommend the Qiagen DNeasy blood and tissue kit for all types of sample volumes.

19. ***AutoGen QuickGene-Mini80 DNA Purification System***: The AutoGen QuickGene-Mini80 DNA purification system was a filter-based extraction method that employed an electric air supply instead of centrifugation to force samples through the filter. The lysing procedure took 90 min of the total 2 h sample-processing time. Although more than eight samples could be in the lysing step at one time, the apparatus used for the washing and eluting steps could only process eight samples at a time. However, the Mini80 system was very efficient

once the lysing procedure was complete. As with other filter methods, the QuickGene filter could become clogged if the cell concentration was too high or the sample was too dirty. One user stated, "The QuickGene Mini80 is a unique filter extraction system that allows for processing larger sample volumes by continually adding to the filter easily."

20. ***QuickSilver Lincoln Nucleic Acid Kit (LiNK) 2.1 Complete Kit***: The QuickSilver LiNK 2.1 DNA extraction kit was only capable of processing one sample at a time, and each sample required approximately 6 min to complete. Operators must hold the sample on the kit for the duration of the 5 min lysing period, which was a negative finding for this kit. The extraction kit was very easy to use, and instructions came enclosed with every individually packaged kit. When only a few samples needed to be analyzed, this extraction method was ideal for a quick PCR-ready sample. Larger volume samples would need to be concentrated because this kit could only process small volumes. A user commented regarding ease-of-use, "This kit is not efficient but does have benefit in its simplistic approach."

## 3.4 Sample-Preparation Study Conclusions

The purpose of this study was to evaluate 20 commercially available nucleic acid extraction kits that could be capable of efficiently purifying DNA from both a Gram-positive organism (*B. atrophaeus* var. *globigii*) and a Gram-negative organism (*Y. pestis* CO92 [pgm–]). These purification processes should allow for the successful PCR identification of the selected target organisms. Additional studies were performed to analyze the DNA purification abilities of these kits when the target organism (*B. atrophaeus* var. *globigii*) was mixed with varying matrices (e.g., sand or clay). The extraction kits were evaluated by general criteria such as cost per sample, processing times, and number of samples that could be processed at once. In addition, technical criteria that were specific to the target organism tested were considered. These included the amount of DNA recovered, the purity of DNA recovered, and the ability of the recovered DNA to be used for successful PCR. End-user reviews on each kit were provided.

### 3.4.1 General Purification Kit Criteria

The following criteria were evaluated for each of the 20 nucleic acid extraction kits that were included in this study:

- *Cost per Sample*: The cost per sample is a common measurement used in acquisition and sustainment investigations. As shown in Table 2, the QuickGene-Mini80 system was the least expensive to operate at $0.30 per sample. As expected, the most-common commercial DNA purification kits (that also generally do not require special equipment) had costs per sample in the $3.00 or lower range. These kits included the Bio-Rad InstaGene Matrix ($0.71 per sample), Promega Wizard kit ($1.80 per sample), Epicentre MasterPure kit ($2.55 per sample), MoBio UltraClean kit ($2.74 per sample), and Qiagen DNEasy blood and tissue kit ($3.30 per sample). A relatively new and less-commonly used system, the CUBRC DNAPro extraction pipette, had a very low cost of $1.00 per sample. Other kits and systems that had per-sample costs in the $4.00 to $10.00 range generally required additional

equipment and may have needed enzyme reagents (these requirements reflected the need for specific sample types and/or DNA extraction processes). These mid-range price kits included the MP FastDNA spin ($3.75 per sample), QIAamp stool ($4.30 per sample), Epicentre SoilMaster ($4.40 per sample), MoBio Powersoil ($4.98 per sample), Akonni TruTip ($5.17 per sample), MP FastDNA spin kit for soil ($5.30 per sample), and bioMerieux NucliSens ($5.50 per sample) kits. The kits for single- or few-sample processing tended to be the most expensive per extraction including the QuickSilver LiNK 2.1 ($59.00 per sample), BioFire IT 1-2-3 Platinum Path ($12.70 per sample), and the Claremont PureLyse ($7.50 per sample) kits.

- *Processing Times*: The sample-processing times for these DNA extraction kits ranged from as short as 5 min to as long as 225 min per test. The Claremont PureLyse gDNA extraction kit had the shortest processing time of 5 min, but this kit was only capable of processing a single sample at a time. This was also the case for the QuickSilver LiNK 2.1 and the IT 1-2-3 Platinum Path sample-purification kits, with extraction times of 15 min. These kits would be effective for the rapid processing of only a few samples. If multiple samples need to be prepared, there are other, more time-effective preparation kits available. For this situation, it may be more time-effective to use one of the following kits: the Bio-Rad Instagene Matrix, the MP FastDNA spin kit, or the MPFastDNA spin kit for soil. All of these kits were capable of processing 22 samples in 30–40 min. For reference, the Promega Wizard Genomic DNA purification kit had the longest processing time of approximately 155–225 min. Also, the recommendations stated here were based only on the processing times for the samples and not on the overall kit performance, which is discussed in Sections 3.4.2 and 3.4.3.

- *Maximum Number of Samples*: Many of the kits tested in this study were capable of processing multiple samples at once (Table 2). In fact, 11 of the 20 kits tested could be used to process up to 22 samples at once. These included Bio-Rad InstaGene Matrix, Epicentre MasterPure Gram-positive DNA purification kit, Epicentre SoilMaster DNA extraction kit, MoBio UltraClean microbial DNA isolation kit, MoBio PowerSoil DNA isolation kit, Molzym PrestoSpin D Bug, MP FastDNA spin kit, MP FastDNA spin kit for soil, Promega Wizard Genomic DNA purification kit, QIAamp stool kit, and Qiagen DNeasy blood and tissue kit. Therefore, these high-throughput kits could be easily used when several samples needed to be processed at once. Four of the kits tested could be used to process only a single sample per test run. These included Claremont PureLyse gDNA extraction kit, CUBRC DNAPro extraction pipette, and the QuickSilver LiNK 2.1 complete kit. These low-throughput kits may be useful for processing low numbers of collected samples in a rapid fashion.

### 3.4.2 Studies Involving a Gram-Positive Organism (*B. atrophaeus* var. *globigii*)

The following criteria were evaluated with the selected Gram-positive organism, *B. atrophaeus* var. *globigii*, using the nucleic acid extraction kits that were included in this study:

- *DNA Purity*: There was large variation among the $A_{260}/A_{280}$ ratios for all of the kits tested against *B. atrophaeus*. Nine of the kits, or 45% of those tested, failed to produce DNA within the targeted ratio of 1.8/2.0 under any of the three conditions examined (10 µg of DNA or $10^7$ or $10^9$ spores). In fact, none of the kits produced DNA within the targeted range for all three categories. The Epicenter MasterPure Gram-positive DNA purification kit, the Epicenter SoilMaster DNA extraction kit, and the QuickGene-Mini80 DNA purification system were the only kits used to obtain positive results for two of the three conditions examined (10 µg of DNA and $10^9$ spores). Using the Claremont Purelyse gDNA and the Molzym PrestoSpin D Bug kits produced positive results with $10^7$ spores. Finally, the Agencourt Genfind v2 DNA, the MoBio UltraClean, the Promega Wizard Genomic DNA purification, and the Qiagen DNeasy blood and tissue kits were all used to produce the targeted ratio recovery when 10 µg of *B. atrophaeus* DNA was processed.

- *DNA Recovery*: All 20 of the selected kits were evaluated for the ability to be used to recover purified DNA from pre-isolated DNA (10 µg) or $10^7$ or $10^9$ spores from *B. atrophaeus*. In these sample sets, there was little consistency among the different isolation methods tested. For the isolated DNA study, the Qiagen DNeasy blood and tissue kit was used to produce the best results, with approximately 55% recovery of DNA. Effectiveness dropped considerably for all kits when we attempted to isolate DNA from spores. The QuickGene-Mini80 DNA isolation kit produced the best results when used to process $10^7$ spores, providing approximately 30% DNA recovery. From $10^7$ spores, the Claremont PureLyse gDNA purification and Agencourt Genfind v2 DNA isolation systems had approximately 25 and 20% DNA recoveries, respectively. Finally, the QuickSilver LiNK 2.1 produced the best results when used to isolate DNA from $10^9$ spores with approximately 15% DNA recovery.

- *PCR Results*: The most important goal for the use of these nucleic acid isolation kits is to produce PCR-ready DNA. Ideally, these data should match the DNA purity ($A_{260}/A_{280}$ ratio) data. In this case, only one kit, the Promega Wizard Genomic DNA purification kit, failed to produce positive PCR results for purifications of $10^7$ and $10^9$ spores. For each sample tested, the remaining kits produced Ct values in the <40 range. The disconnection between PCR results and purity by absorbance likely reflects the carryover of buffer components that skew absorbance at 280 or 260 nm but have little impact on PCR analysis.

### 3.4.3 Studies Involving a Gram-Negative Organism (*Y. pestis* CO92 [pgm–])

The following criteria were evaluated with the selected Gram-negative organism, *Y. pestis* CO92 (pgm–), using the nucleic acid extraction kits that were included in this study:

- *DNA Purity*: There was a large amount of variation among the $A_{260}/A_{280}$ ratios for all of the kits tested against *Y. pestis* CO92 (pgm–). Nine of the kits (45% of those tested) failed to produce DNA within the targeted ratio of 1.8/2.0 when used under any of the three conditions examined (10 µg of DNA or $10^7$ or $10^9$ vegetative cells). Only the Epicentre MasterPure Gram-positive DNA purification kit showed successful purification of the DNA in terms of $A_{260}/A_{280}$ ratio when used for all three conditions examined. Four kits (Akonni TruTip microbial DNA kit, MoBio UltraClean, MP FastDNA spin kit for soil, and Qiagen DNeasy blood and tissue kit) were used for successful purifications with the 10 µg of DNA and $10^9$ vegetative cells tests. The Promega Wizard Genomic DNA purification kit and the QuickGene-Mini80 DNA purification system were successfully used with $10^9$ vegetative cells. Finally, the Agencourt Genfind v2 DNA kit and the IT 1-2-3 Platinum Path sample-purification kit were used for successful purification runs when examining 10 µg of *Y. pestis* DNA.

- *DNA Recovery*: Compared with the studies using *B. atrophaeus*, the studies that attempted to recover DNA from *Y. pestis* showed much more consistency and better performance among the various DNA isolation kits tested. Two of the kits, the Promega Maxwell 16 and the Qiagen DNeasy blood and tissue kit, had >70% recovery when used to purify previously isolated *Y. pestis* DNA. All of the kits examined were used to recover at least some amount of measurable DNA during this test. When attempting to isolate DNA from $10^7$ vegetative cells, six of the kits produced ≥50% DNA recovery. These kits included the Akonni TruTip microbial DNA, the Agencourt Genfind v2 DNA, the bioMerieux NucliSens miniMAG, the Bio-Rad InstaGene Matrix, MP FastDNA spin kit for soil, and Qiagen DNeasy blood and tissue kit. In fact, two of these kits (Agencourt Genfind v2 DNA and Qiagen DNeasy blood and tissue) were used to obtain ≥85% recovery of the *Y. pestis* vegetative cell DNA. When testing a concentration of $10^9$ cells, four of the kits displayed ≥40% DNA recovery (Agencourt Genfind v2 DNA, MoBio Power Soil, MP FastDNA spin kit for soil, and Qiagen DNeasy blood and tissue kit). In this situation, the Qiagen DNeasy blood and tissue kit was used to produce the best results (>45% DNA recovery).

- *PCR Results*: Like the previous portion of this study, the DNA purity results ($A_{260}/A_{280}$ ratios) did not align with the PCR results. For the *Y. pestis* study, only one kit, the MP FastDNA spin kit, failed to produce positive PCR results for any of the conditions examined. All of the other kits examined in this study reported Ct values of <40 for all of the *Y. pestis* samples tested.

### 3.4.4 Studies Involving Varying Test Matrices

In this portion of the study, we examined the effect of different sample matrices (clay and sand) on the purification of *B. atrophaeus* spores. For these studies, clay or sand was spiked with either $10^7$ or $10^9$ spores and processed for DNA purification with five different nucleic acid isolation kits including: Bio-Rad Instagene Matrix kit, Epicentre SoilMaster DNA extraction kit, IT 1-2-3 Platinum Path sample-purification kit, MoBio PowerSoil DNA isolation kit, MP FastDNA spin kit for soil, and QIAamp stool kit. In terms of isolating purified DNA from the different matrices, only the Epicentre SoilMaster DNA extraction kit was used to produce DNA with an acceptable $A_{260}/A_{280}$ ratio from $10^7$ spores spiked into the clay matrix. Although this result was in the acceptable range, the SEM was rather large. Three of the five kits examined provided positive PCR results for both matrices examined in this study. The Epicentre SoilMaster DNA extraction kit, the IT 1-2-3 Platinum Path sample-purification kit, and the MoBio PowerSoil DNA isolation kit were used to produce positive results for the detection of *B. atrophaeus* ($10^7$ or $10^9$ spores) in clay and sand. Overall, using the MoBio PowerSoil DNA isolation kit provided the lowest Ct values of these kits for all of the conditions tested. The QIAamp stool kit worked well when used with spores mixed with sand samples, but not with samples mixed with clay. Finally, using the Bio-Rad Instagene Matrix and the MP FastDNA spin kit for soil did not produce any positive PCR results with the clay or sand samples tested.

## 4. LABORATORY ASSESSMENT (SAMPLE COLLECTION)

### 4.1 Sample-Collection Study Materials and Methods

The laboratory assessment portion of this study included specific devices, materials, and procedures for sample collection, which are described in Sections 4.1.1 through 4.1.5.

### 4.1.1 Sample-Collection Devices

For the sample-collection portion of this study, six individual sample-collection devices were evaluated. These devices included the BiSKit (biological sampling kit; QuickSilver Analytics, Inc.), SRK (swab rinse kits), Environmental Swab Systems (Copan Diagnostics, Inc.; Murrieta, CA), Backpack Surface Extractor (InnovaPrep, LLC; Drexel, MO), M-Vac (Microbial-Vac Systems, Inc.; Bluffdale, UT), and the S2P (swab sampling powder) and S3 kits (QuickSilver Analytics, Inc.). A summary of the collection devices that were evaluated are shown in Table 7. Of the collection devices used in this study, four were manually operated, and two were automatically operated. Samples were collected according to the manufacturers' recommended instructions.

**Table 7. Manufacturer and Collection Device List**

| Manufacturer | Collection Device | Type of Collection |
|---|---|---|
| QuickSilver Analytics, Inc. | BiSKit | Swab |
| Copan Diagnostics, Inc. | Copan SRK | Swab |
| InnovaPrep, LLC | IP Backpack Surface Extractor | Vacuum |
| Microbial-Vac Systems, Inc. | M-Vac | Vacuum |
| QuickSilver Analytics, Inc. | QS S2P kit | Swab |
| QuickSilver Analytics, Inc. | QS S3 | Swab |

### 4.1.2 Reference Materials

For the sample-collection study, *B. atrophaeus* var. *globigii* spores obtained from the Critical Reagents Program were used. The surface coupons selected for this study included glass, stainless steel, sealed concrete, finished wood, and carpet. The sample collections were performed in triplicate and processed in accordance with the manufacturers' instructions to determine organism concentration and viable organism recovery as appropriate (Section 4.1.3).

### 4.1.3 Quantitative Viable Organism Evaluation

After samples were collected, viable organisms were evaluated in triplicate by plating the samples on the appropriate solid culture medium, incubating the plates overnight, and using an automated colony counter to obtain data. Results were reported as the mean ± SEM of the number of colony-forming units recovered per milliliter.

### 4.1.4 Nucleic Acid Extraction

Nucleic acid extraction for this portion of the study was performed using the Epicentre MasterPure Gram-positive DNA extraction kit according to the manufacturer's recommended instructions.

### 4.1.5 Nucleic Acid Evaluation

The recovered nucleic acids were evaluated as stated in Section 3. The collected samples were evaluated for DNA purity, percentage of DNA recovery, and effectiveness of RT-PCR identification.

## 4.2 Sample-Collection Study Results

Viable bacteria were retrieved after collection from multiple surfaces as shown in Figure 2.

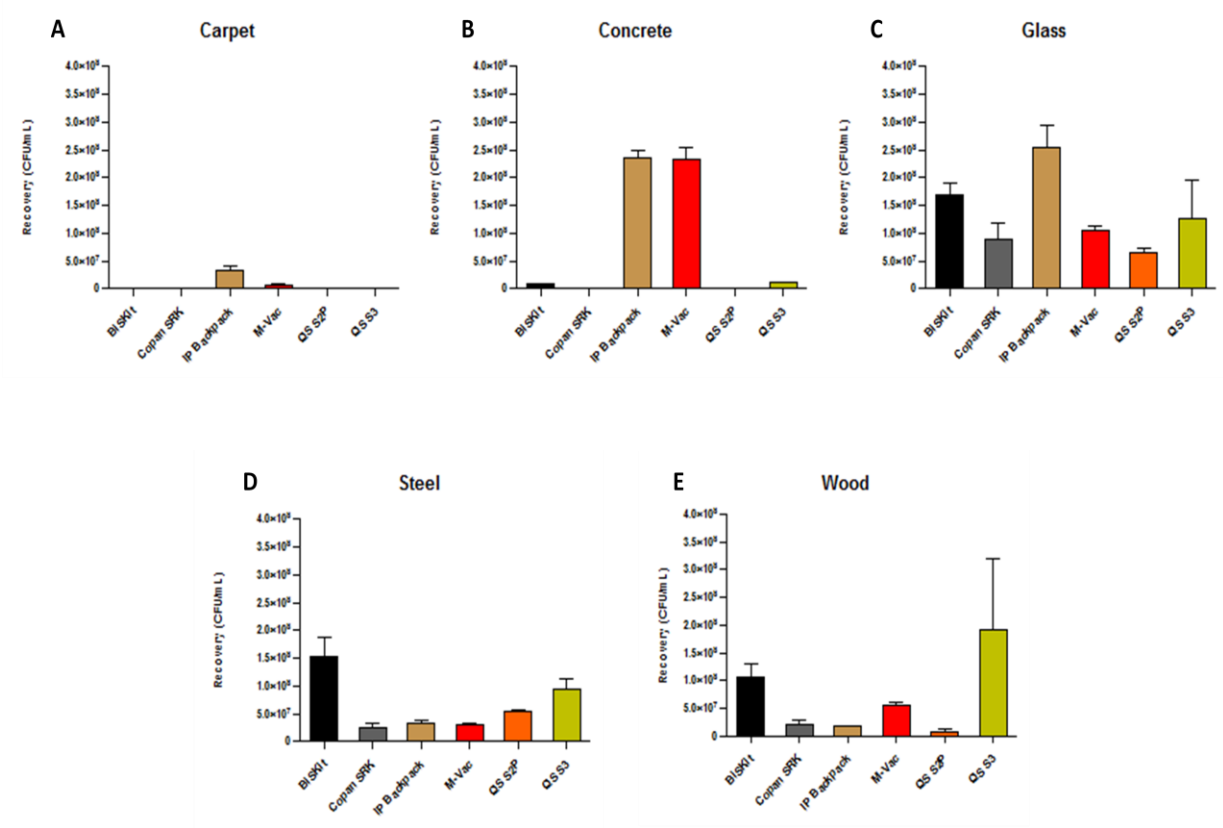


**Figure 2. Average amount of bacteria recovered after collection from multiple surfaces.**

Figure 2 shows five different surfaces, including carpet (A), concrete (B), glass (C), steel (D), and sealed wood (E), which were treated with $10^9$ *B. atrophaeus* spores and then collected using the devices described in Section 4.1.1. The bar graphs shown in Figure 2 depict the devices in various colors as follows: BiSKit (black), Copan SRK (gray), IP Backpack (tan), M-Vac (red), QS S2P (orange), or QS S3 (green). The collected samples were plated on agar plates, placed in an incubator at 37 °C for 24 h, and then the viable colonies were counted to determine the bacterial concentration. All of the sample-collection devices were capable of being used to retrieve viable bacteria from glass, steel, and sealed wood. Recovering viable samples from carpet proved to be the most difficult. Only two collection devices (IP Backpack and M-Vac) were able to recover viable bacteria from carpet. Concrete was also difficult to efficiently sample; only four of the six collection devices had the ability to recover measurable viable bacteria from concrete. Overall, the best recovery occurred when a glass surface was sampled. The IP Backpack was the most efficient sample-collection device, with a recovery of approximately $2.5 \times 10^8$ cfu of viable bacteria/mL, and the QS S2P was the least efficient device, with a recovery of approximately $5.0 \times 10^7$ cfu of viable bacteria/mL.

Overall, the M-Vac system was capable of collecting DNA from all of the tested surfaces that had an appropriate $A_{260}/A_{280}$ ratio or were within one SEM of this range (Table 8). The QS S2P device performed well in this test on three (carpet, steel, and wood) of the five surfaces, and the BiSKit device performed well in this test on two (glass and wood) of the five surfaces tested.

**Table 8. Average Purity of DNA Extraction Performed after Collection from Multiple Surfaces**

| Surface | Collection Device | $A_{260}/A_{280}$ Ratio |
|---|---|---|
| Carpet | BiSKit | 3.14 ± 0.50 |
| | Copan SRK | 1.43 ± 0.09 |
| | IP Backpack | 1.25 ± 0.73 |
| | M-Vac | 1.93 ± 0.26 |
| | QS S2P | 1.98 ± 0.12 |
| | QS S3 | 2.30 ± 0.33 |
| Concrete | BiSKit | 2.71 ± 0.32 |
| | Copan SRK | 1.43 ± 0.03 |
| | IP Backpack | 4.13 ± 4.90 |
| | M-Vac | 1.65 ± 0.15 |
| | QS S2P | 2.90 ± 0.18 |
| | QS S3 | 2.04 ± 0.29 |
| Glass | BiSKit | 1.75 ± 0.05 |
| | Copan SRK | 1.37 ± 0.09 |
| | IP Backpack | 3.77 ± 3.51 |
| | M-Vac | 1.75 ± 0.14 |
| | QS S2P | 2.54 ± 0.46 |
| | QS S3 | 1.67 ± 0.18 |
| Steel | BiSKit | 2.14 ± 0.23 |
| | Copan SRK | 1.39 ± 0.04 |
| | IP Backpack | –0.40 ± 0.58 |
| | M-Vac | 1.96 ± 0.15 |
| | QS S2P | 2.18 ± 0.21 |
| | QS S3 | 1.70 ± 0.05 |
| Wood | BiSKit | 1.75 ± 0.08 |
| | Copan SRK | 1.43 ± 0.06 |
| | IP Backpack | 0.58 ± 0.66 |
| | M-Vac | 1.73 ± 0.04 |
| | QS S2P | 2.24 ± 0.40 |
| | QS S3 | 1.72 ± 0.07 |

Purified *B. atrophaeus* var. *globigii* DNA was obtained after collection of samples from multiple surfaces as shown in Figure 3.

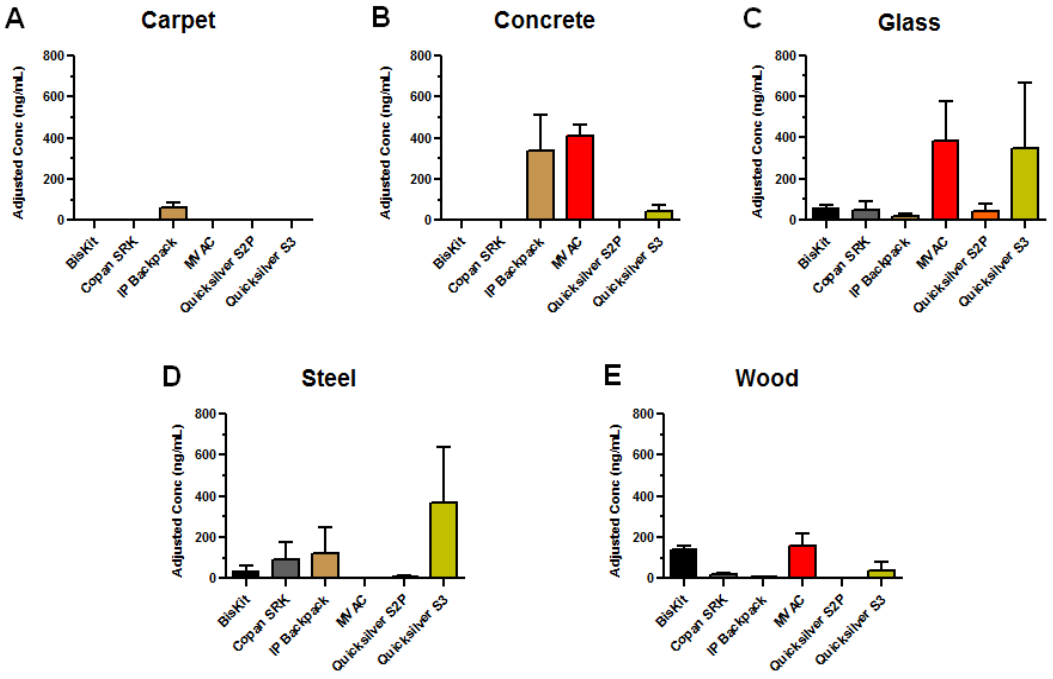


**Figure 3. Average amount of purified *B. atrophaeus* var. *globigii* DNA obtained after collection from multiple types of surfaces.**

Figure 3 shows five different surfaces, including carpet (A), concrete (B), glass (C), steel (D), and sealed wood (E), which were treated with $10^9$ *B. atrophaeus* spores and then collected using the devices described in Section 4.1.1. The bar graphs shown in Figure 3 depict the devices in various colors as follows: BiSKit (black), Copan SRK (gray), IP Backpack (tan), M-Vac (red), QS S2P (orange), or QS S3 (green). All of the collection devices showed the ability to recover measurable amounts of DNA from glass (C). Five of the six collection devices were used to recover measurable DNA from both steel (D) and wood (E). Measurable DNA was collected from concrete (B) using three of the six collection devices. Collecting measurable DNA from carpet (A) proved to be the most difficult. Only the IP Backpack device could be used to recover enough DNA for successful measurement with the PicoGreen assay.

The QS S3 device was able to provide positive PCR results (Ct $\leq$ 40) for *B. atrophaeus* with each of the surfaces tested (Table 9). The BiSKit and the Copan SRK devices both provided positive PCR results (Ct < 40) for all but one of the surfaces tested (carpet with BiSKit and steel with Copan SRK). The M-Vac and the QS S2P devices were unable to provide positive PCR results for collections from any of the surfaces tested (i.e., Ct values were undetermined).

**Table 9. Average Ct after Collection from Multiple Surfaces**

| Surface | BiSKit | Copan SRK | IP Backpack | M-Vac | QS S2P | QS S3 |
|---|---|---|---|---|---|---|
| Carpet | 43.61 | 37.08 ± 0.62 | 25.94 ± 0.08 | Undetermined | Undetermined | 33.96 ± 0.50 |
| Concrete | 32.22 ± 1.93 | 33.21 | 26.25 ± 0.56 | Undetermined | Undetermined | 28.93 ± 0.52 |
| Glass | 33.11 | 26.77 ± 1.59 | 26.36 ± 0.35 | Undetermined | Undetermined | 26.31 |
| Steel | 32.58 ± 3.02 | Undetermined | 27.67 ± 0.78 | Undetermined | Undetermined | 25.08 ± 0.17 |
| Wood | 25.75 ± 0.59 | 25.42 ± 0.07 | 27.62 ± 0.78 | Undetermined | Undetermined | 29.65 ± 3.25 |

## 4.3 Sample-Collection Study Conclusions

The purpose of the sample-collection portion of this study was to evaluate the effectiveness of six different collection systems for their ability to provide PCR-ready DNA from *B. atrophaeus* spores. The spores were collected from five different surfaces (carpet, concrete, glass, steel, and sealed wood). Two of the collection systems, the IP Backpack Surface Extractor and the M-Vac, were vacuum-based collection systems. Four of the collection systems, the BiSKit, Copan SRK, QS S2P, and QS S3, were swab-based systems. All of the collection devices were evaluated on the following parameters: the amount of live organisms recovered, the amount of DNA recovered, the purity of DNA recovered, and the potential for downstream applications (e.g., PCR) using the collected DNA.

In the initial part of this study, examination of each collection device revealed varying effectiveness in the recovery of viable bacteria from each surface tested. All six collection devices were able to recover measurable amounts of viable bacteria from three of the five surfaces tested (glass, steel, and sealed wood). Testing with glass resulted in the greatest recovery of viable bacteria. Carpet proved the most difficult surface to successfully sample. Only two of the devices (IP Backpack and M-Vac) were able to be used to recover any measurable amount of bacteria, but the efficiencies for these devices were very low. In general, the vacuum-based collection devices appeared to have the most flexibility for successful collection from all of the surfaces tested.

Each collection system displayed varying amounts of effectiveness depending on the surface examined. All six collection devices were capable of collecting measurable amounts of DNA from the glass surface (Figure 2C). The M-Vac and the QS S3 devices were able to collect the largest amounts of DNA from glass (~400 ng of DNA/mL). Five of the six collection devices were used to provide measurable amounts of DNA from the steel (Figure 2D) and wood (Figure 2E) surfaces. The QS S3 had the best collection ability on steel surfaces, with an approximately 400 ng of DNA/mL recovery. The BiSKit and M-Vac devices were the most effective on wood surfaces (~150 ng of DNA/mL). On the concrete surface (Figure 2B), the IP Backpack, the M-Vac, and the QS S3 devices were used to recover measurable amounts of DNA, and the M-Vac device performed the best (~400 ng of DNA/mL). Collecting measurable DNA from carpet (Figure 2A) proved to be the most difficult. Only the IP Backpack device was capable of recovering measurable amounts of DNA (~50 ng/mL) from carpet.

Overall, the IP Backpack performed the best for sample collection—it was the only device that collected measurable amounts of DNA from each surface tested. The QS S3 device effectively collected DNA from four of the five surfaces. The BiSKit, the Copan SRK, and the M-Vac effectively collected measurable amounts of DNA from three of the five surfaces, and the QS S2P effectively collected DNA from only two of the five surfaces tested.

Much of the DNA collected in this study did not pass the purity threshold ($A_{260}/A_{280}$ ratio of 1.8/2.0). Of all the conditions tested, only the M-Vac and the QS S2P devices were able to produce any positive results in this category. Spores collected from carpet and steel provided the only pure DNA obtained using the M-Vac device. Spores collected from carpet provided the only pure DNA obtained using the QS S2P device. Even though the samples collected using the IP Backpack or QS S3 devices did not produce any DNA that was within the purity threshold, the samples collected with these devices displayed positive PCR results ($Ct \leq 40$) for all surfaces examined (Table 9). The BiSKit collected PCR-capable DNA from all of the surfaces tested except carpet ($Ct = 43.61$), and the Copan SRK device obtained PCR-ready DNA from all surfaces except steel (Ct was undetermined). The M-Vac and the QS S2P devices were unable to produce any PCR results for DNA collected on any of the surfaces tested.

# 5. LABORATORY ASSESSMENT (SAMPLE PRESERVATION)

## 5.1 Sample-Preservation Study Materials and Methods

The laboratory assessment portion of this study included specific materials and methods for sample preservation and storage, which are described in Sections 5.1.1 through 5.1.4.

### 5.1.1 Reference Materials

For the sample-preservation study, *Y. pestis* CO92 (pgm–) vegetative cells were selected as the target organism. The bacterial stock used in this study was obtained from the Critical Reagents Program.

### 5.1.2 Sample-Preservation Materials

In this study, four different commercially available sample-preservation materials and devices were used (Table 10). These included the AssayAssure universal environmental collection kit (Thermo Fisher Scientific; Tewksbury, MA), the DNAgard ambient temperature DNA stabilizer (Biomatrica, Inc.; San Diego, CA), Whatman FTA* cards (GE Healthcare Life Sciences; Pittsburgh, PA), and the HemaSpot blood-sampling device (Spot On Sciences, Inc.; Austin, TX).

* FTA: fast technology analysis of nucleic acids

**Table 10. Manufacturer and Preservation Method List**

| Preservation Method | Manufacturer | Preservation Type |
|---|---|---|
| AssayAssure Universal Environmental Collection Kit | Thermo Fisher Scientific, Inc. | Liquid |
| DNAgard Tissues and Cells | Biomatrica, Inc. | Liquid |
| Whatman FTA cards | GE Healthcare Life Sciences | Paper |
| HemaSpot Blood-Sampling Device | Spot On Sciences, Inc. | Paper |

### 5.1.3 Nucleic Acid Evaluation

The recovered nucleic acids were evaluated as stated in Section 3. The experimental samples were evaluated for DNA purity, percentage of DNA recovery, and effectiveness of RT-PCR identification.

### 5.1.4 Quantitative Viable Organism Evaluation

After samples were preserved, viable organisms were evaluated in triplicate by plating the preserved samples on the appropriate solid culture medium, incubating the plates overnight, and using an automated colony counter to obtain data. Results were reported as the mean ± SEM of the colony-forming units recovered per milliliter.

## 5.2 Sample-Preservation Study Results

*Y. pestis* samples were collected and then placed in AssayAssure, DNAgard, and HemaSpot preservation methods and stored at 4 or 25 °C for 1 or 2 days, shown in Figures 4A and B, respectively. The samples were then examined for bacterial viability. All of the preservation processes provided bacterial viability at a concentration of $\geq 1.0 \times 10^{10}$ cfu/mL after 1 day of storage at either 4 or 25 °C (except AssayAssure at 25 °C, shown in Figure 4A). After 2 days of storage, there were significant reductions in bacterial viability for all processes examined (Figure 4B). Biomatrica's DNAgard method stored samples at 4 °C and performed the best, with a recovery of >1000 cfu of viable bacteria/mL. Whatman FTA paper was not included in this study because no recovery of viable bacteria occurred at any time point examined (data not shown).

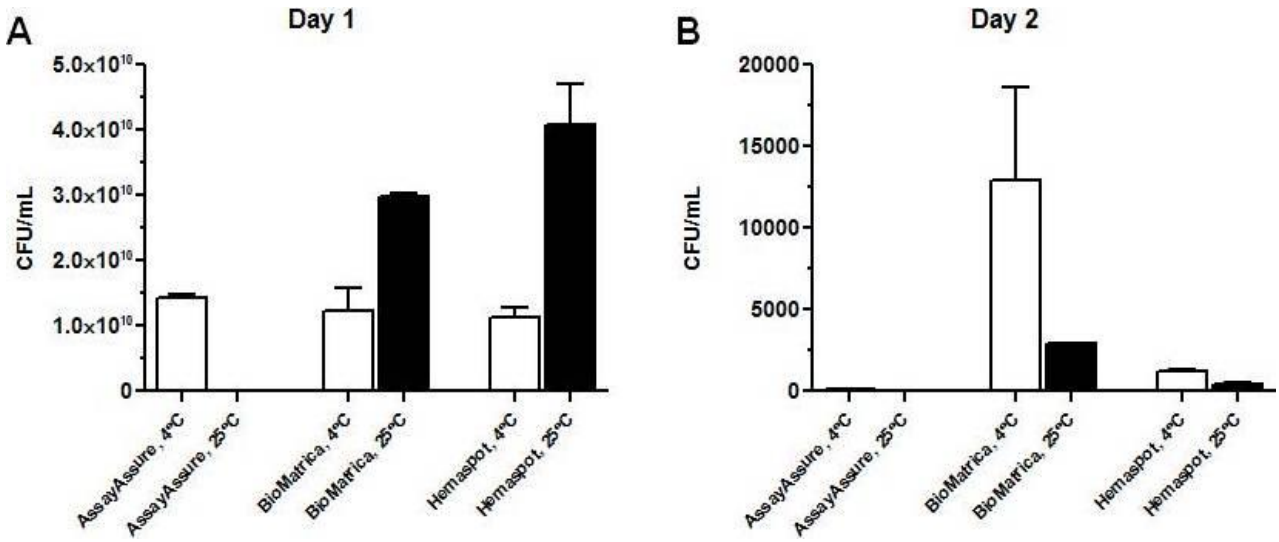


**Figure 4. Preservation of viable *Y. pestis* CO92 (pgm–) at 4 and 25 °C on Day 1 (A) or Day 2 (B).**

Samples that were stored at increasing temperatures (4, 25, 37, and 50 °C) for 0–14 days were examined for resultant DNA purity by analyzing the $A_{260}/A_{280}$ ratio (Table 11). The samples stored with the DNAGard method gave the best results with this test. Ninety-five percent of those samples examined had DNA within an acceptable range of $A_{260}/A_{280}$ ratio of 1.8/2.0. The amount of samples yielding results within the acceptable range for the FTA and AssayAssure methods included 75 and 55%, respectively. Finally, using the HemaSpot method for preservation provided no samples within the acceptable range.

**Table 11. Comparison of the Average Purity of DNA Extraction Obtained after Using DNA Preservation Methods for *Y. pestis* CO92 (pgm–) Vegetative Cells**

| Method | Day | $A_{260}/A_{280}$ at Tested Storage Temperatures | | | |
|---|---|---|---|---|---|
| | | 4 °C | 25 °C | 37 °C | 50 °C |
| AssayAssure | 0 | 1.62 ± 0.09 | 1.73 ± 0.03 | 1.72 ± 0.03 | 1.70 ± 0.02 |
| | 1 | 1.66 ± 0.02 | 1.66 ± 0.02 | 1.58 ± 0.03 | 1.66 ± 0.01 |
| | 2 | 1.91 ± 0.02 | 1.93 ± 0.02 | 1.81 ± 0.00 | 1.85 ± 0.04 |
| | 6 | 1.85 ± 0.02 | 1.94 ± 0.02 | 1.74 ±0.02 | 1.87 ± 0.10 |
| | 14 | 1.93 ± 0.01 | 1.90 ± 0.01 | 1.79 ± 0.01 | 1.83 ± 0.01 |
| DNAGard | 0 | 1.91 ± 0.01 | 1.88 ± 0.01 | 1.80 ± 0.01 | 1.68 ± 0.07 |
| | 1 | 1.91 ± 0.01 | 1.91 ± 0.01 | 1.91 ± 0.01 | 1.91 ± 0.003 |
| | 2 | 2.03 ± 0.03 | 2.03 ± 0.05 | 1.97 ± 0.01 | 1.96 ± 0.02 |
| | 6 | 1.97 ± 0.03 | 1.94 ± 0.02 | 2.00 ± 0.01 | 1.94 ± 0.02 |
| | 14 | 2.02 ± 0.03 | 1.97 ± 0.02 | 1.89 ± 0.003 | 2.01 ± 0.02 |
| FTA | 0 | 1.69 ± 0.03 | 1.85 ± 0.17 | 1.89 ± 0.16 | 1.80 ± 0.07 |
| | 1 | 2.26 ± 0.38 | 1.88 ± 0.07 | 1.81 ± 0.04 | 1.82 ± 0.003 |
| | 2 | 1.84 ± 0.03 | 1.89 ± 0.03 | 1.86 ± 0.02 | 1.86 ± 0.02 |
| | 6 | 2.07 ± 0.04 | 2.20 ± 0.07 | 1.99 ± 0.03 | 2.15 ± 0.04 |
| | 14 | 1.87 ± 0.01 | 2.03 ± 0.08 | 1.85 ± 0.03 | 1.96 ± 0.03 |
| HemaSpot | 0 | 1.61 ± 0.04 | 1.58 ± 0.03 | 1.60 ± 0.07 | 1.41 ± 0.02 |
| | 1 | 1.45 ± 0.01 | 1.65 ± 0.01 | 1.68 ± 0.01 | 1.48 ± 0.01 |
| | 2 | 1.70 ± 0.03 | 1.65 ± 0.01 | 1.64 ± 0.03 | 1.61 ± 0.04 |
| | 6 | 1.73 ± 0.03 | 1.72 ± 0.03 | 1.70 ± 0.04 | 1.62 ± 0.04 |
| | 14 | 1.63 ± 0.04 | 1.59 ± 0.003 | 1.63 ± 0.02 | 1.61 ± 0.04 |

The concentration of DNA recovered using each preservation method was measured using the PicoGreen assay (Figure 5). For the AssayAssure (A) and DNAgard (B) methods, 400 µL of *Y. pestis* sample was added to the liquid preservative. For the Whatman FTA (C) and HemaSpot (D) methods, 100 µL of *Y. pestis* sample was added to the paper-based preservative devices. In the AssayAssure (A) and DNAgard (B) experiments, recovered DNA concentrations were relatively consistent over the times and temperatures examined. With the exception of the initial high concentrations at Day 0, the HemaSpot (D) device provided consistent concentrations of recovered DNA. The Whatman FTA (C) paper had the lowest amount of recovered DNA of all the preservation methods tested, but the results were relatively consistent over the times and temperatures examined.

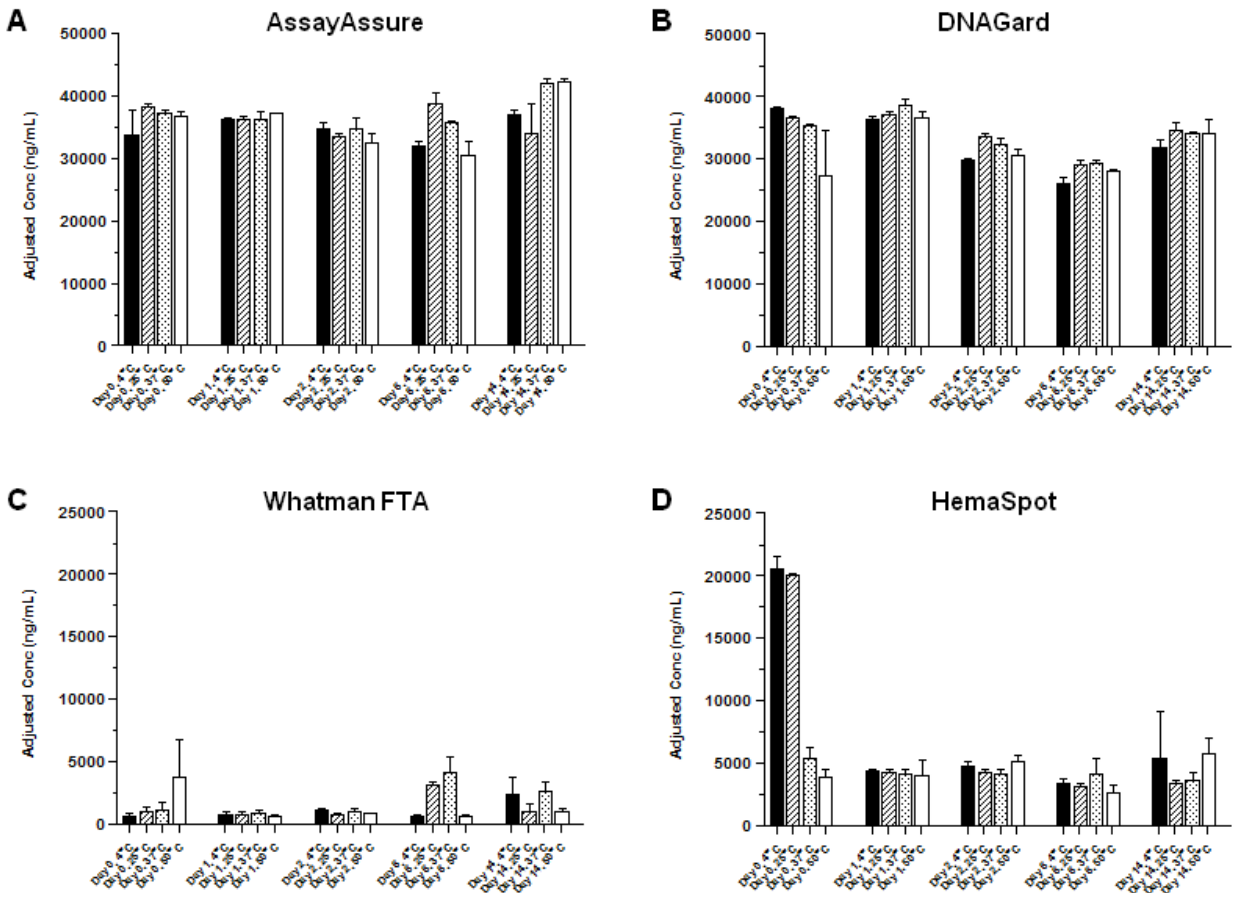


**Figure 5. Average concentration of *Y. pestis* CO92 (pgm–) DNA recovered with each DNA preservation method.**

In this test, the FTA preservation method was the most effective for the recovery of *Y. pestis* CO92 (pgm–) DNA after storage, even though it was not capable of preserving live bacteria. Of a total of 20 different environmental conditions, using the FTA method resulted in successful PCR analyses in 19 of 20 experimental conditions (Table 12). The HemaSpot method was the second most effective, with 8 out of 20 successful PCR analyses. The DNAgard method only produced two successful PCR analyses, while the AssayAssure method was unsuccessful for all PCR attempts.

**Table 12. Average Ct of PCR Analysis Performed on DNA Recovered from Each DNA Preservation Method**

| Method | Day | Ct at Tested Storage Temperatures | | | |
|---|---|---|---|---|---|
| | | 4 °C | 25 °C | 37 °C | 50 °C |
| AssayAssure | 0 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 1 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 2 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 6 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 14 | Undetermined | Undetermined | Undetermined | Undetermined |
| DNAgard | 0 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 1 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 2 | Undetermined | 23.12 ± 0.10 | Undetermined | 24.18 ± 1.28 |
| | 6 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 14 | Undetermined | Undetermined | Undetermined | Undetermined |
| FTA | 0 | 22.25 ± 0.22 | 22.28 ± 0.43 | 23.10 ± 0.89 | Undetermined |
| | 1 | 23.32 ± 0.52 | 22.74 ± 0.43 | 22.76 ± 0.06 | 22.42 ± 0.19 |
| | 2 | 23.19 ± 0.25 | 23.07 ± 0.36 | 26.05 ± 1.59 | 24.33 ± 0.48 |
| | 6 | 24.48 ± 0.77 | 25.11 ± 1.93 | 24.41 ± 0.90 | 24.29 ± 0.83 |
| | 14 | 26.49 ± 2.66 | 25.22 ± 1.50 | 23.98 ± 0.74 | 25.72 ± 2.69 |
| HemaSpot | 0 | Undetermined | Undetermined | Undetermined | Undetermined |
| | 1 | Undetermined | 20.76 ± 0.08 | 21.58 ± 0.04 | Undetermined |
| | 2 | 21.63 ± 0.13 | 21.72 ± 0.02 | 21.86 ± 0.03 | Undetermined |
| | 6 | 22.02 ± 0.16 | 21.90 ± 0.10 | Undetermined | 22.40 ± 0.21 |
| | 14 | Undetermined | Undetermined | Undetermined | Undetermined |

## 5.3 Sample-Preservation Study Conclusions

The purpose of the preservation portion of the study was to evaluate the effectiveness of four different preservation methods (liquid- and paper-based) in preserving viable bacteria as well as PCR-ready DNA for an extended time period under increasing temperatures. After samples were stored under the test conditions, all preservation methods were

evaluated on the basis of plate counts of live organisms, amount of DNA retained, purity of the retained DNA, and successful PCR analysis of the retained DNA.

Collected samples of *Y. pestis* were initially evaluated for the retention of viable cells after 1 or 2 days of storage at 4 or 25 °C (Figure 3). Storage of bacteria using the AssayAssure, DNAgard, or HemaSpot methods for 1 day at 4 °C resulted in the retention of approximately $1.0 \times 10^{10}$ cfu/mL for all three preservation methods. After 1 day of storage at 25 °C, the DNAgard and HemaSpot methods were able to retain approximately $3.0 \times 10^{10}$ and $4.0 \times 10^{10}$ cfu/mL, respectively. After 2 days of storage, the AssayAssure method was used to retain a barely detectable amount of viable bacteria (<1000 cfu/mL) at 4 °C and no bacteria at 25 °C. The DNAgard method had the best performance at 2 days with the retention of approximately 12,000 and 2,500 cfu/mL at 4 and 25 °C, respectively. The HemaSpot method retained approximately 1000 and 500 cfu/mL at 4 and 25 °C, respectively. Finally, the FTA paper was unable to be used to preserve any viable bacteria at any time it was examined. Given the short amount of time before cell viability would be lost (1–2 days), these preservation methods would not be recommended for use in the retention of viable bacteria for an extended time period.

All four of the preservation methods performed well in terms of retention of DNA. Consistent results for DNA concentrations were observed for testing with the time periods of 0, 1, 3, 6, and 14 days and with temperatures of 4, 25, 37, and 50 °C. It appeared that little-to-no DNA degraded. The samples were also evaluated for DNA purity by examining the $A_{260}/A_{280}$ ratio. Of the four methods tested, Biomatrica's DNAgard method performed the best in this assay, with 18 of 20 samples observed within the acceptable range of 1.8–2.0. The FTA paper was the second-best method in terms of DNA retention, with 14 of 20 samples falling within the acceptable range. Using the AssayAssure method produced 10 of 20 samples within the acceptable range, but using the HemaSpot method produced no samples within this range. Unfortunately, these DNA purity results did not correlate with successful PCR analysis results. Even though the DNAgard method produced pure DNA according to the $A_{260}/A_{280}$ ratio, only 2 of the 20 samples examined gave positive PCR results. Use of the FTA paper produced the best results in terms of retaining PCR-ready DNA; only 1 of the 20 samples tested gave an undetermined result, and all of the positive PCR results resulted in Ct values in the 22–26 range. The HemaSpot method, which performed poorly in the $A_{260}/A_{280}$ ratio measurements, had 8 of 20 positive PCR results. Finally, use of the AssayAssure method of preservation provided no positive PCR results.

## 6. FINAL CONCLUSIONS

The studies detailed in this report showed that there were several commercial products that could effectively perform one of the three critical steps of sample processing. However, an ideal sample-processing kit that encompassed all three steps was not identified. The effectiveness of the commercial products often depended on the targeted organism, the surface type that was sampled, and the temperature and time between sample collection and delayed sample preparation.

Considering those caveats, several commercial products were shown to effectively collect deposited bacteria from various surface types. For nonporous surfaces, all commercial products effectively collected bacteria for later growth and/or identification. However, for the porous concrete and carpet surfaces, only the vacuum devices were efficient in the collection of deposited bacteria. For sample preservation, the Biomatrica DNAgard solution and the AssayAssure universal environmental collection kit buffer effectively preserved bacterial DNA for 14 days before nucleic acid isolation and PCR analysis were performed. The Biomatrica DNAgard solution was also used to preserve bacteria for cultivation for more than 48 h after collection. The HemaSpot device showed marginal effectiveness in preserving bacterial DNA over 14 days, and the FTA paper was not an effective preservative. The most-effective bacterial DNA preparation products ranged from single-use items (i.e., Akonni TruTip microbial DNA and Claremont PureLyse kits) to relatively inexpensive, moderate throughput kits that required little or no ancillary equipment (i.e., MoBio UltraClean and Qiagen DNEasy blood and tissue kit) to reagents and equipment combinations that were relatively expensive (i.e., Promega Maxwell 16 and QuickGene-Mini80 systems). Knowledge of potential collection surfaces, possible target organisms, and probable sample-holding conditions before DNA preparation are all important considerations when making effective choices of commercial sample-processing kits and equipment.

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# ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| BiSKit | biological sampling kit (QuickSilver Analytics device) |
| Ct | cycle threshold |
| DNA | deoxyribonucleic acid |
| ECBC | U.S. Army Edgewood Chemical Biological Center |
| FAM | 6-carboxyfluorescein |
| FTA | fast technology analysis (of nucleic acids) |
| IEC | International Electrochemical Commission |
| ISO | International Organization for Standardization |
| JPEO-CBD | Joint Program Executive Office for Chemical Biological Defense |
| JPM-MCS | Joint Project Manager Medical Countermeasure Systems |
| JPM-TMT | Joint Project Manager Transformational Medical Technologies |
| N/A | not applicable |
| PCR | polymerase chain reaction |
| pgm– | pigmentation negative |
| PMP | paramagnetic particle |
| RT-PCR | real time-polymerase chain reaction |
| S2P | swab sampling powder (InnovaPrep, LLC) |
| SEM | standard error of the mean |
| SRK | swab rinse kits (Copan Diagnostics device) |
| TAMRA | tetramethylrhodamine |
| TE buffer | tris-ethylenediaminetetraacetic acid buffer |
| UCC | Unified Culture Collection |
| USG | U.S. Government |


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